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MYCOLOGIA

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VOL. LIII MAY–JUNE, 1961 NO. 3

NEW SPECIES AND REVISIONS IN THE ORDER TUBERALES

HELEN MARGARET GILKEY

(WITH 3 FIGURES)

Myrmecocystis spinospora Gilkey, sp. nov. FIG. 1

Ascocarpus quoad cognitus plusminusve 6 mm diametro, albus recens, conspicue convolutus, hymenio cum loborum extremitatibus congruenti sed saepe interrupto; asci ab coartatione plusminusve contorti; sporae fuscae, sphaericae, 26–32.5 μ (caelato incluso), spinosae, spinis non confertis, invalidis, distincte liberis, ad basim latissimis, 3.5–4 μ longitudine.

Ascocarp, as known, averaging 6 mm in diam, white when fresh, conspicuously convolute, the hymenium following the contour of the lobes but frequently interrupted; asci more or less contorted by crowding; spores dark, globose, 26–32.5 μ (including sculpturing), spinose, the spines uncrowded, weak, distinctly free, broadest at the base, 3.5–4 μ long.

Virginia, 10 miles south of Charlottesville, 3–4 inches below the soil surface, in mixed woods; D. W. Bierhorst, TYPE (CUP 43847, Cornell; portion, 867 HMG).

This interesting fungus, submitted by Dr. R. P. Korf, reinforces a growing recognition of a definite relationship between *Myrmecocystis* and *Genabea*. So close has become the known linkage between these hitherto readily recognizable genera that perhaps eventually no dividing line can reasonably be retained. This situation has independently been discussed by Fischer (**3**, p. 14) and Gilkey (**4**, p. 10), and further discoveries since those dates support this view.

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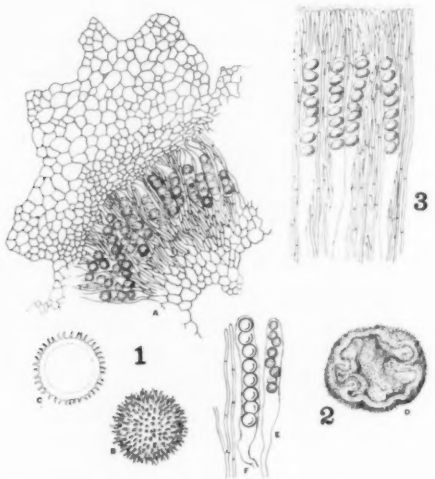
As at present understood, *Myrmecocystis* and *Genabea* are separated from *Genea* by the complete absence of a mycelial tuft; and, for the most part (*M. compacta* (Hark.) Gilkey is an exception), by conspicuous progressive sterilization of the hymenium by means of intrusions and cohesions of folds, with the extreme result in *Genabea* of separate fertile pockets opening to the surface at several points. In *Myrmecocystis,* as thus far known, such sterilization though conspicuous is less extreme, leaving the basic form still traceable, and the openings frequently single.



FIGS. 1–3. 1. *Myrmecocystis spinospora.* A. Portion of hymenium. B. Spore. C. Optical section of spore. 2. *Hydnocystis Singeri.* D. One-half an ascocarp. E. Ascus with immature spores. F. Mature ascus and spores. 3. *Hydnoplicata Whitei.* Portion of hymenium.

In the present species, increasing complexity in the hymenial area is evident, but the basic structure and form are still essentially those of *Myrmecocystis* rather than *Genabea.*

Hydnocystis Singeri Gilkey, sp. nov. FIG. 2

Ascocarpus plusminusve epigaeus, omnino clusus, 1 cm aut minus diametro, subglobosus, prope planus aut tenuiter sulcatus, 'Dresden Brown' (R) siccus, minute puberulus, sine coniunctione cum mycelio basali sed pristini tomenti velantis testatus; gleba alba, cavo simplice aut ex parte diviso per insolitis dissepimentis coniunctis e parietibus interioribus hymenio vesititis; hyphae glebae plusminusve 6.5 μ latitudine, reticulum intricatum formantes plusminusve confertim confluentes

prope ad superficiem, corticem prosenchymatis pseudoprosenchymatisque formantes; asci 190–228 μ longitudine, octospori; sporae sphaericae, leves, 21 μ vel plerumque minores diametro; paraphyses paulum quam asci breviores, simplices vel ramosae.

Ascocarp more or less epigaeous, completely closed, 1 cm or less in diam, subglobose, nearly even or shallowly furrowed, Dresden Brown (R) when dry, minutely puberulent, without basal mycelial attachment but with evidence of an original enveloping tomentum; gleba white, cavity simple or with partial partitioning accomplished by occasional fused intrusions from the hymenium-lined inner walls; hyphae of the gleba averaging 6.5 μ in width, arranged in a complex network, this becoming more or less fused and compact near the surface, forming a cortex of prosenchyma or pseudoparenchyma; asci 190–228 μ long, 8-spored; spores globose, smooth, 21 μ or generally less in diam; paraphyses averaging somewhat shorter than the asci, simple or branched.

Rio de los Sosas, alt. 1000 m; Dr. Rolf Singer, TYPE (Singer T 2144; portion, 860 HMG).

This new species introduces certain compounding of the glebal area hitherto unknown in *Hydnocystis*. However, the closed ascocarp, the hymenial structure and arrangement, and the smooth spores, belong to this genus; and the introduction of the present species results in no more pronounced glebal variation than is already known in other members of the Tuberales such as *Geopora* and *Hydnotrya*.

The closed simple form of fruiting body and the determinate paraphyses separate this fungus from *Stephensia* with which it shares a pubescent surface and smooth spores (similarly globose in most species of the latter genus). Another genus characterized by such spores and asci is *Phymatomyces*, a monotypic genus known only from a single collection made in Japan (**8**). The ascocarp of the single species, *P. yezo-montanus* Kobayasi, is described and figured, however, as *Hydnotrya*-like, with internal cavities opening at several points to the surface, thus excluding ours. Unfortunately there is no opportunity of making a first-hand comparison of the two since, according to Dr. Kobayasi, the only collection was destroyed by bombing during World War II.

HYDNOPLICATA

Since publication of this new genus from Australia (**6**), additional material received not only from the type locality in New South Wales but from several other provinces as well, has necessitated slight revision of the original concept of the genus, though without alteration of its status as distinct from other genera of the Tuberales. The following description should be substituted for the original;

Ascocarp lobed, provided with a more or less conspicuous mycelial tuft, the lobes and internal folds of the ascocarp forming canals and chambers opening generally at several points to the surface, and lined by a palisade of asci and paraphyses; asci cylindrical, paraphyses longer than the asci, separate, but held in close association by a gelatinous matrix; spores minutely roughened, ellipsoid, uniseriate.

Since the paraphyses, though held together by a gelatinous sheath (FIG. 3), are found not to be fused at their tips into a pseudoparenchymatous epithecium, the genus is not appropriately placed in the family Geneaceae, but may legitimately belong to Tuberaceae, near *Hydnotrya* in which a similar structure is sometimes suggested. The portion of the key to genera in Tuberaceae (TUBERALES, N. Am. Flora II 1: 9. 1954) which is involved in this discussion, may be modified as follows:

Spores sculptured
Ascocarp without mycelial tuft.....2. HYDNOTRYA
Ascocarp with mycelial tuft (known only in Australia)....HYDNOPLICATA

The key to genera in Geneaceae (ibid., p. 3), from which *Hydnoplicata* is now excluded, should read:

Mycelial tuft present; opening in ascocarp single; asci not conspicuously crowded; spores smooth or sculptured, ellipsoid to rarely subglobose, never globose
Spores smooth.....1. PETCHIOMYCES
Spores sculptured.....2. GENEA
Mycelial tuft none; openings in ascocarp one to several; asci generally crowded, often distorted; spores sculptured, ellipsoid or globose
Ascocarp rarely nearly even to generally conspicuously lobed, hollow, the hymenium continuous or more or less interrupted; spores globose.....3. MYRMECOCYSTIS
Ascocarp compact, the hymenium interrupted by sterile tissue, occurring as isolated fertile areas embedded in pseudoparenchyma; spores (in ours) ellipsoid.....4. GENABEA

DENSOCARPA

Since its establishment in 1954 (**5**), this genus is found to have an apparently close affinity to *Stephensia sumatrana*, described by Dr. K. B. Boedijn from Indonesia (**1**); and a less obvious relationship to the type species of the genus, *S. bombycina* (Vitt.) Tul. (**10**, p. 130) of Europe (*Genea bombycina* Vitt.) (**11**, p. 9).

Although in neither the American nor the Sumatran specimens can be found the basal or central cavity conspicuously figured and described in literature for *S. bombycina*, the casual evidence of vein convergence at base of the ascocarp slightly suggests a similar morphological history;

and the smooth globose spores in typically cylindrical asci, accompanied by indeterminate paraphyses, are characteristic of all three species.

The universal tomentum described for *Stephensia* generically, probably exists in *Densocarpa*, also, but only as a juvenile character. Its early presence is indicated by occasional remnants, particularly at the base where what appears to be a loosely-defined mycelial tuft can be seen. But in none of the many ascocarps examined was a complete floccose covering found.

Odor, though generally a questionable character upon which to judge relationships, sometimes offers a clue and may be useful here. Of *Stephensia bombycina*, Dr. Lilian Hawker (7) describes the odor as "strong, unpleasant, of drains or overripe Camembert cheese"; Dr. Boedijn quotes the collector of *S. sumatrana* as saying "the fresh fructifications emitted a strong smell of H_2S"; while Dr. Alex. Smith says of *Densocarpa Shanori*, "It is strong, exactly like sewer gas."

In order to accommodate *Densocarpa* within its limits, the genus *Stephensia* is emended below; and a slightly revised description of *Densocarpa Shanori*, under the new combination, is presented.

STEPHENSIA Tul. emend. Gilkey

Stephensia Tul., Fungi Hypog. 129. 1851.

Fruiting body variously shaped, enclosed at least when young in a universal tomentum; gleba with veins or narrow open or hypha-filled cavities more or less obviously radiating from the base or from a basal or central open cavity, the cavities and venae externae lined by the hymenium; asci cylindrical or slightly clavate, 8-spored at first, in some species only 1–4 spores maturing; spores globose, smooth; paraphyses indeterminate in length, some branching beyond the asci and partially filling the narrow cavities.

Stephensia Shanori (Gilkey) Gilkey, comb. nov.

Densocarpa Shanori Gilkey, N. Am. Flora II **1**: 16. 1954; Mycologia **46**: 786, *f. 1–5.* 1954 [1955].

Ascocarp hypogaeous to partially epigaeous at maturity, fleshy, firm, nearly even above, slightly lobed beneath, color Light Pinkish Cinnamon (R) to Ferruginous (R), the upper surface more or less tessellate from shallow cracking, with occasional evidence of an early-evanescent tomentum, especially at the base; odor disagreeable; gleba whitish, penetrated by veins and small cavities showing casual radiation from the base of the ascocarp; asci short-cylindrical or slightly clavate; spores 16.5–19.5 μ, only 1–4 maturing; paraphyses forming a hyphal web within each hymenium-lined cavity, the web often breaking away before maturity.

DISTRIBUTION: Illinois: Urbana; Michigan: Ann Arbor.

Six species have now been assigned to *Stephensia*. One of these, *S. crocea* Quélet, was reduced by Dr. Ed. Fischer (**3**) to a variety of *S. bombycina*. A second, *S. arenivaga* Cooke & Massee (**2**), taken in Australia, is found to be completely unlike *Stephensia* in ascocarp characters. The four remaining species may be keyed as follows:

Spores long-ellipsoid [Reported (**9**) as a narrowly-limited endemic in Europe; unknown to the present author] ..... *S. Peyronelii*
Spores globose
  Ascocarp permanently tomentose; mature spores typically 8 to an ascus
    Venae externae communicating with a well-defined basal (and often a central) cavity in the ascocarp (known only from Europe) ..... *S. bombycina*
    Venae externae somewhat obscurely converging at base of ascocarp; neither basal nor central cavity present (known only from Sumatra) ..... *S. sumatrana*
  Ascocarp not permanently tomentose; mature spores 1–4 to an ascus (known only from America) ..... *S. Shanori*

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To the many persons from five continents and from several island groups who have generously supplied specimens for study and comparison during the preparation of this paper, I wish to express most sincere thanks.

CORVALLIS, OREGON

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A RE-EVALUATION OF ARACHNIOTUS INDICUS

Gouri Rani Ghosh, G. F. Orr and H. H. Kuehn

(with 5 figures)

Chattopadhyay and Das Gupta (**1959**) repeatedly isolated a relatively simple ascomycete from soil of paddy fields in Chinsurah, West Bengal, India. A culture of the fungus was sent to the Commonwealth Mycological Institute, Kew, England, where it was determined to be a new species of *Arachniotus*. Among the several strains isolated by Chattopadhyay and Das Gupta representing this new species there was one isolate which had sufficiently different cleistothecial characters to merit its description as a variety. These investigators subsequently described the new species as *Arachniotus indicus* and its variety as *A. indicus* var. *major*.

A cultural description of *A. indicus* was given as it appeared on only one medium, potato-dextrose agar. Cleistothecia were light orange-yellow and measured 162–520 μ diam. Peridial hyphae were described as weblike and formed an imperfect peridium with peridial cells thin-walled and orange-yellow. Ascospores were oval to globose, pale orange-yellow and measured 3.2–4 $\times$ 2.8–3.6 μ, averaging 3.6 $\times$ 3.2 μ.

The variety, *A. indicus* var. *major*, differed from *A. indicus* in having larger cleistothecia which measured 227–1000 μ diam and which were bright yellow initially but later became brown. Also, the peridial wall was somewhat more loosely arranged than in *A. indicus*.

Through the courtesy of Dr. S. B. Chattopadhyay we were able to obtain cultures of *A. indicus* and its variety. Our study of these isolates revealed several salient features which evidently had been overlooked by previous investigators, and it is our intent in the present paper to report such findings.

Further studies have shown that the fungi in question, *Arachniotus indicus* and *A. indicus* var. *major*, are not representatives of the Gymnoascaceae, but are strains of *Talaromyces* (*Penicillium*) *vermiculatus* (Dang.) C. R. Benjamin. Benjamin (**1955**) described the genus *Talaromyces* to include the ascosporic penicillia included in the *P. luteum* series by Raper and Thom (**1949**).

FIG. 1. *Arachniotus indicus.* Two mature ascocarps. × 315.

A conidial state is present (FIG. 5) in which conidia are produced on sterigmata in a manner characteristic of the genus *Penicillium.* The sterigmata are lanceolate-tapered in the manner characteristic of the genus *Talaromyces.* Ample conidial production is easily obtained on certain media rich in nitrogen rather than carbon. Sectoring frequently

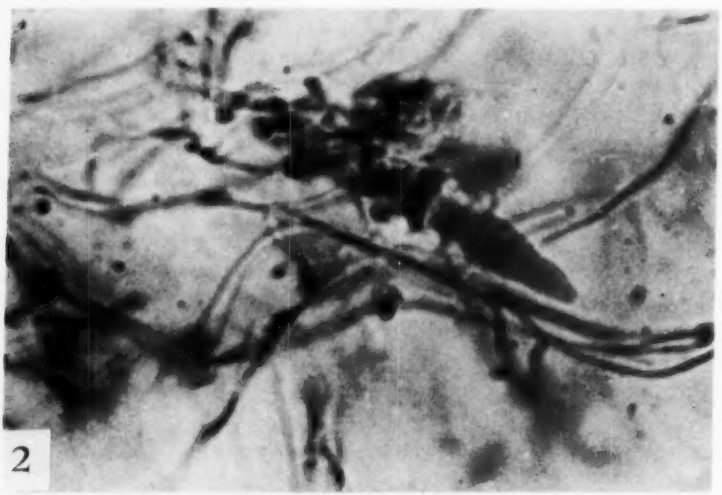

FIG. 2. *Arachniotus indicus.* Gametangial coil with the slender antheridium coiling about the central, stout ascogonium. × 1590.

occurs on Freezing Agar (Kuehn, Orr and Ghosh, **1961**) and the conidiophores vary from fragmentary to typically biverticillately symmetrical. Subcultures on other media exhibit varying amounts of conidial production. It is true that the penicillus as seen in FIG. 5 is rather fragmentary, but this condition often is present in some strains of *Talaromyces vermiculatus*. In regard to this species, Raper and Thom (**1949**, p. 580) stated, "penicilli produced in varying numbers in different strains, ranging from fragmentary to typical biverticillately symmetrical. . . ."

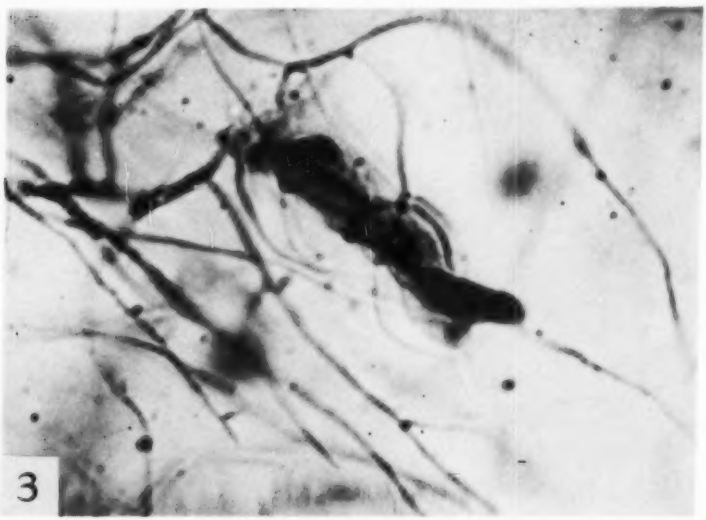

FIG. 3. *Arachniotus indicus* var. *major*. Gametangial coil showing what appears to be fusion at the apices where the two initials touch. × 1300.

Kuehn (**1957**) discussed the occurrence of conidial stages of *Arachniotus* species as reported in the literature, and claimed that with two exceptions, *Byssochlamys nivea* Westling and *B. fulva* Olliver & Smith, no known gymnoascaceous fungi produce conidia. He further maintained that *Byssochlamys* should be considered to be a simple representative of the Eurotiaceae. *Gymnoascus flavus* Klocker was reported by Klocker (**1902**) to form conidia, but these undoubtedly were chlamydospores. *Arachniotus trisporus* Hotson was described as producing conidia on penicillate structures, but this fungus actually represents *Byssochlamys nivea*. Thus, it would seem that not a single carefully

studied species of *Arachniotus,* nor in fact of the entire family Gymnoascaceae, is known to produce a penicillate imperfect stage.

It might be well here to review the relation of *Byssochlamys* to *Pseudoarachniotus.* Both genera lack discrete ascocarps, but arthospores or aleuriospores, or both, are present in *Pseudoarachniotus* while conidia on penicillus-like structures (*Paecilomyces*-like) and chlamydospores are produced in *Byssochlamys.* We prefer to restrict the Gymnoascaceae to fungi which lack conidia borne on phialides (sterigmata) and produce only aleuriospores, arthrospores or chlamydospores. In fact, some species completely lack an imperfect state. In our concept the Gymnoascaceae increase in complexity from simple forms lacking discrete ascocarps (*Pseudoarachniotus*) through forms

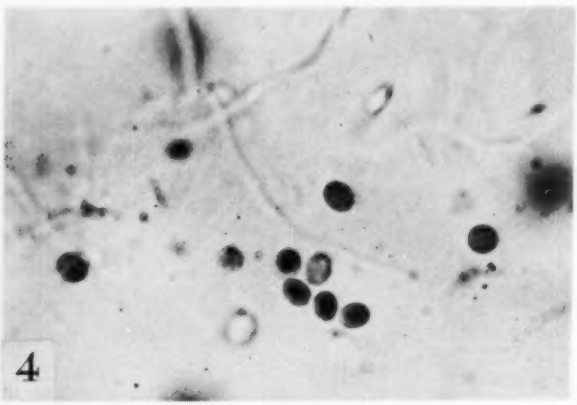

FIG. 4. *Arachniotus indicus.* Mature ascospores showing the delicate echinulations. × 1470.

with undifferentiated netlike peridia (*Arachniotus*) to forms with differentiated netlike peridia (*Gymnoascus, Myxotrichum, Ctenomyces*).

The gametangial coil formed by *A. indicus* and its variety is shown in FIGS. 2 and 3, which illustrate the coil in different focal planes. The initials consist of a long, thick, unbranched clublike ascogonium around which there is coiled a much thinner antheridium. FIG. 3 shows what appears to be fusion of antheridium and ascogonium. Marked similarity can be seen to the illustration of Raper and Thom (**1949**, p. 568, Fig. 144B). Those investigators stated that the initials of *Penicillium vermiculatum* were regarded to be a distinguishing characteristic in the identification of that species.

The cleistothecium of *A. indicus* (FIG. 1) and its variety is quite simple and its peridium is merely a network of unmodified, interwoven hyphae. As such, it is perhaps somewhat similar to the type of ascocarp usually associated not only with the genus *Arachniotus* but also with the genus *Talaromyces*. Benjamin (**1955**) made the observation that "the whole genus *Talaromyces* would seem to be composed of a series of forms intermediate between the lower members of the Gymnoascaceae and the Eurotiaceae." He also stated that *Talaromyces luteus* appeared to be "almost as closely related to the genus *Arachniotus* of the Gymnoascaceae as to some of the other members of *Talaromyces*." Raper and Thom (**1949**, p. 573) also recognized the affinities of these two groups

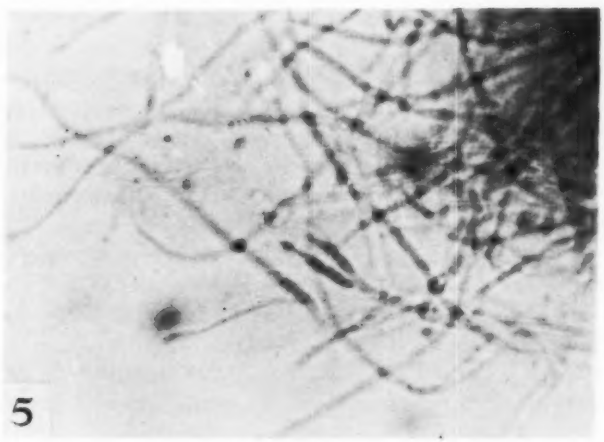

FIG. 5. *Arachniotus indicus*. A typical, fragmentary penicillus. × 1000.

of fungi and they reviewed the controversy regarding this situation. They felt, as do the writers, that all forms with penicillate structures should remain together as a taxonomic group. Therefore, it is not difficult to find precedent for past difficulty in the proper identification of fungi belonging to this complex of fungi. Nevertheless, the key characters separating the Gymnoascaceae from the Eurotiaceae would appear to be the absence of the usual type of conidial formation in the Gymnoascaceae in conjunction with other characters, especially the anastomosis pattern of the peridial weft as opposed to the usually complete enclosure of the asci by a peridium in the Eurotiaceae.

Ascospores of *A. indicus* and its variety are elliptical, pale orange-

yellow and finely echinulate over the entire surface (FIG. 4). Again, this description corresponds to that given for *Talaromyces vermiculatus* (Raper and Thom, **1949**, p. 582).

In *A. indicus* and its variety the penicillate conidial state is almost completely absent and penicilli are not easily seen on potato-dextrose agar, Czapek's agar, and certain other media. This is characteristic of the genus *Talaromyces* as a whole on such media, and evidently this situation has led to incorrect identifications in the past. For example, Emmons (**1935**) studied two strains of *T. vermiculatus* including one received from Dr. Westerdijk as *Arachniotus ruber* van Tieghem. Also, Saccardo (**1895**) transferred *Penicillium* (*Talaromyces*) *luteus* to *Gymnoascus* because of the lack of a discrete ascocarp. However, his decision was based upon bibliographic considerations rather than upon a study of material of *P. luteus*.

The variation in size of ascocarps of *A. indicus* and its variety as given by Chattopadhyay and Das Gupta (**1959**) poses no problem in the identification of these strains as *T. vermiculatus*. Raper and Thom (**1949**, p. 582) stated that ascocarps of this species vary greatly in size in different strains or even in the same strain, but *commonly* measure 200–500 μ diam. Thus, the measurements for *A. indicus* (162–550 μ) and for its variety (227–1000 μ) fall within an acceptable range of variation. Raper and Thom gave the usual ascospore size as 4–4.5 × 3–3.5 μ, but in some strains spores measured up to 5.2 μ long. One strain had spores which measured 6–6.5 × 2–4.5 μ. Ascospore measurements given by Chattopadhyay and Das Gupta were 3.2–4 × 2.8–3.6 μ (average 3.6 × 3.2 μ) and would represent those of a small-spored *T. vermiculatus*.

SUMMARY

Type strains of *Arachniotus indicus* and *A. indicus* var. *major* were examined. Both isolates proved to represent *Talaromyces* (*Penicillium*) *vermiculatus* (Dang.) C. R. Benjamin. The re-identification was based on the presence of a penicillate conidial state. In addition, both isolates possessed gametangial initials and ascocarp and ascospore characters closely associated with *T. vermiculatus*.

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NOTES ON BOLETES. XIV

WALTER H. SNELL AND ESTHER A. DICK

The following observations and studies, of new or old material, were made in the course of activities prosecuted with the aid of National Science Foundation Grant 9043.

BOLETUS GLABELLUS

Since Peck described this species in 1888, its status has been uncertain. There are records of its collection at least twice in Wisconsin, and we have occasionally in the Northeast come across what we thought was probably this species. The first of these, collected at Deerhead, N. Y., in 1932, appeared to fit Peck's description and to match the type material. We have been a little less certain with regard to a few other collections from the middle western and northeastern states, but a single specimen obtained in Sandwich, N. H., in 1959 is the second with regard to which we entertain no doubts. Consequently, we are restoring *B. glabellus* Peck to good standing and placing it in the Section Luridi because of its small pores.

BOLETUS ALUTACEUS

Peck published the description of this species of Morgan's from Kentucky. In the summer of 1960, near a birch, a hamamelis bush and some oaks on the Kancamagus Highway out of Conway, N. H., Dick made a collection which fits the description so closely, almost to the letter, that we have no alternative to calling it this species. The surface is in general subglabrous and only slightly innately fibrillose or subtomentose in places, but the trama appears definitely to be phylloporoid and not at all bilateral. Therefore, we are placing it in the section Subtomentosi of *Xerocomus*, as **Xerocomus alutaceus** (Morgan in Peck) Dick & Snell, comb. nov. (basionym: *Boletus alutaceus* Morgan in Peck, Bull. N. Y. State Mus. **8**: 109. 1889).

BOLETUS CALOPUS

We are confident that a collection made in South New Berlin, N. Y., in 1932 is *Boletus calopus* Fr.

BOLETUS SEPARANS

Singer made *B. separans* Peck a subspecies of *B. edulis* Bull. ex Fr. (9, p. 26, 27) for reasons which appeared to him to be sufficient, and perhaps also as supported by the statement of Coker and Beers (1, p. 35) that intergrading forms occur. Singer stated, however, that if there should be a constant difference in color of the spore-prints, there would be good reason for specific distinction.

The fresh spore-print of *B. edulis* and its subspecies is definitely deep olivaceous (Olive Brown). The fresh spore-prints of the entity *B. separans* that we have obtained are brownish-ochraceous or ochraceous-brown, without any tinge of olive; Coker and Beers say—"with little or no olive."

We compared spore-prints that were about 25 years old. The prints of *B. edulis* are now a slightly deeper Dresden Brown, with faint tinge of olivaceous; the old prints of *B. separans* are a slightly more ochraceous Dresden Brown, a little towards Buckthorn Brown. In other words, even the old prints retain to a certain degree the differences in the fresh prints—the print of *B. edulis* towards the olive and that of *B. separans* still with ochraceous hue.

We believe *B. separans* Peck to be an autonomous species, and Singer (correspondence) is inclined to agree.

SUILLUS SIBIRICUS

In 1960, Dick made a collection in La Vérendrye Park, Province of Quebec, of a bolete with most of the outward characteristics of *Suillus sibiricus* (Singer) Singer, except that it lacked an annulus. Inasmuch as the Quebec collection was made under rather dry conditions and the annulus of *S. sibiricus* is fugaceous, it was concluded that the dry condition may have been responsible for its disappearance. A comparison of the Quebec collection with material (Farlow Herbarium) labelled authentic for *S. sibiricus* revealed that the only discernible differences were the lack of an annulus and somewhat smaller pores (our specimen is smaller); a comparison of the spores, however, raised some questions.

In one of the collections, by Cain from Ontario, the spores are as given in the original description—broadly fusiform-ellipsoid, 3.5–5 μ broad. In the other collections, from farther west in Canada, the spores are slightly shorter, mostly more ellipsoid but some fusiform at one end and few like those of Cain's collection, and measure 3–4 μ broad, mostly 3–3.3 μ. The spores of our Quebec collection are like those of this latter group. In all cases, the spores were from the fruit-bodies and not from spore-prints.

Cain's collection, with the rather distinctive spores, is typical *S. sibiricus* in all respects and the other available collections differ only in the slightly varying spores. If our Quebec collection is *S. sibiricus*, it is of interest from two points of view. It is the first time, as far as we know, that this species has been collected east of the Province of Ontario. And it was found in an almost pure stand of *Pinus banksiana*.

SUILLUS GRANULATUS SUBSP. SNELLII, S. ALBIDIPES AND S. PLACIDUS

These three taxa have presented problems in the past and even now give difficulties, especially under certain conditions. Singer made the American form of *S. granulatus* (L. ex Fr.) Kuntze, common under *Pinus strobus*, a subspecies—*Snellii* (**6**, p. 40). We made some minor contributions with regard to *S. placidus* (Bon.) Singer in North America (**10**, p. 231; **11**, p. 14). *S. albidipes* (Peck) Singer was never well understood by us until Singer gave a good description (**8**, p. 272, 273), but even now there are some problems remaining, and at all times we find it necessary to be very careful in our attempts at identification. For example, early in 1960, under very dry conditions and several miles apart, we collected two specimens which at first glance we considered to be *S. granulatus* subsp. *Snellii*. After the most careful examination, macroscopic and microscopic, we decided that one specimen was *S. placidus* with atypical, dried glandular dots on the stipe, and the other *S. albidipes*. We have had many other similar experiences.

S. granulatus subsp. *Snellii* and *S. albidipes* are usually similar in stature and colors, or at least show the same variations. *S. placidus* usually has a longer stipe and a pileus that is white or ivory-colored, perhaps with a yellow margin in earlier stages, but can have the same stature and colors as the other two under conditions of age or dryness at least.

The tube-layer is adnate-decurrent in *S. placidus* and most often adnate in *S. granulatus* subsp. *Snellii* and decurrent in *S. albidipes*, but with respect to the latter, the distinction is often not clear.

We are not familiar with a veil in *S. placidus* but one can be found very rarely in *S. granulatus* subsp. *Snellii*, as noted by Singer (**8**, p. 261). *S. albidipes* has a veil which shows on the margin of the pileus in young stages but which cannot be found later.

The stipes of *S. granulatus* subsp. *Snellii* and *S. placidus* are often reticulate for 1 cm below the tubes, but we know of no reticulation ever being mentioned for *S. albidipes*.

The glandular dots on the stipe are usually distinctive. In *S. granulatus* subsp. *Snellii*, they are rather small and abundant on the upper half of the stipe. In *S. albidipes*, they are smaller even to the point

of indistinctness, are scattered, and whereas they may occur halfway down the stipe, they are most often confined to a narrow zone at the very apex. The dots of *S. placidus* are much different—large, closely spaced, often fused to give a pseudoreticulate or speckled-reticulate appearance, in moist weather profusely exuding droplets and under less moist conditions having a waxy feeling. On the other hand, under very dry conditions some degree of care and perspicacity is necessary to discern the difference between the dots of *S. placidus* and *S. granulatus* subsp. *Snellii*.

The spores are as follows:

S. granulatus subsp. *Snellii*—5.5–10 × 2.8–3.2 μ, mostly 7–7.5 × 3± μ, very pale melleous under oil.

S. albidipes—6.8–12 × 2.7–3.7 μ, rarely to 4 μ broad, mostly 7.8–8.7 (or perhaps 9.5) × 3.2–3.3 μ, pale melleous to melleous under oil, with few to many distinctly olivaceous.

S. placidus—(5.5–)6.2–11(–12) × 2.7–3.7(–4) μ, mostly about 8 or 8–9 × 3–3.3 μ, pale melleous.

In other words, if most of the spores are 7–7.5 μ long and about 3 μ broad, and very pale, the specimen is *S. granulatus* subsp. *Snellii*. If most of the spores are over 7.5 μ long and slightly broader than 3 μ, and if there are some spores more or less olive-colored, then the specimen is *S. albidipes*. If there are no olive spores present, *S. albidipes* and *S. placidus* cannot be distinguished with certainty by the spores alone.

All three entities are associated with *Pinus strobus*, *S. placidus* always in our experience and *S. granulatus* subsp. *Snellii* fundamentally so but with the unusual situations already noted by us (**12**, p. 375–377; **2**, p. 444). With regard to *S. albidipes*, Singer (**8**, p. 273) states as follows: "In open coniferous and mixed woods under or near *Pinus strobus* (Sect. Cembrae), but occasionally, in regions where white pine is common, also growing with *Abies balsamea*." We are at present about convinced that *Tsuga canadensis* should be added.

The chemical reactions have received attention by Singer and by us. They are not so important for *S. placidus* but Singer has suggested that slight differences in the reactions of *S. granulatus* subsp. *Snellii* and *S. albidipes* might be of assistance, and for a while we thought we had some distinctions upon which we could rely. Recent continued testing, however, especially with carpophores growing under different conditions and in different stages of development, have given results that are neither so distinctive nor constant. Accordingly, we are still pursuing this study.

From the foregoing discussion, as it relates to *S. granulatus* subsp. *Snellii* and *S. albidipes*, it can readily be understood why Peck first named the latter a variety of *S. granulatus* (**4**, p. 168), although he later changed his mind and made it a species (**5**, p. 22). Singer said (**8**, p. 273) that *S. albidipes* "may eventually be reduced to the status of a subspecies of the latter" (e.g. *S. granulatus*) and this reduction we are now making, as *Suillus granulatus* subsp. **albidipes** (Peck) Snell & Dick, comb. nov. (basionym: *Boletus granulatus* L. ex Fr. var. *albidipes* Peck, Ann. Rep. N. Y. St. Mus. **54**(1): 168. 1901).

A YELLOW BOLETUS

We have always hoped to find and be able to clarify an all-yellow, unchanging bolete that has to our knowledge never been relocated—*Boletus unicolor* Frost in Peck, one of Peck's Viscipelles. We have never found anything we could suspect was this old species, but twice recently in New Hampshire we have collected a form that was not only all-yellow but also upon bruising or cutting turned blue everywhere, without and within. Inasmuch as this appears to be one of the concolorous-pored Luridi, there are only three choices as to its identity—a yellow form of *B. miniato-olivaceus* Frost, a northern form of the Floridian *B. flavissimus* (Murrill) Murrill, or the European *B. junquilleus* (Quélet) Boudier.

We would never have thought of considering these New Hampshire specimens as *B. miniato-olivaceus* if we had not read Singer's discussion (**9**, p. 65) stating that there is an all-yellow form. We have never seen this species without some red or pink. Our specimens under discussion not only do not have any red, but they do not have the disagreeable odor, they differ somewhat in other characters, and in general they do not look quite right for *B. miniato-olivaceus*.

Some of the pertinent descriptive details of *B. flavissimus* may be mentioned. The surface of the pileus is usually covered with a very fugacious, red floccosity which is rarely persistent beyond the earliest stages, is often red-dotted or red-spotted when old, and no bluing is mentioned. In the context, the bluing is not constant and neither is the red at the base of the stipe. The tubes and pores turn blue only "often" or "frequently." The stipe is perhaps glabrous and may be reticulate, is later red at the base and red-dotted or red-lineate higher up, and unchanging or bluing. In our New Hampshire material, to repeat, the bluing is constant all over, outside and inside; there is no red floccosity on the surface; the stipe has no red inside or outside, is not glabrous and is not at all reticulate.

Our specimens meet the requirements of *B. junquilleus* almost per-

fectly, with one or two exceptions. The stipes are more or less strigose-tomentose at the base but not to the extent of making them appear somewhat hairy, and not at all extensively up the stipe. And perhaps most important, the spores are a little narrow. They measure in width 4–5 or 5.5 μ, a few 6 μ, whereas Kallenbach gives the widths as 5–6–6.5 μ, with a few up to 7 μ (**3**, p. 12, for *B. pseudosulphureus* Kallenbach).

It certainly seems to us that our collections are more like *B. junquilleus* than *B. flavissimus* and that they are not *B. miniato-olivaceus* unless there is an all-yellow form in the north as there is in the south, with other differences from the usual form or forms.

TWO LURIDI

A collection from Alaska by Mrs. Virginia Wells has been determined, after as careful study as we can give it, as *Boletus erythropus* (Fr. ex Fr.) Pers. The spores are slightly smaller than the measurements given in European descriptions and found in our European collections, but we can determine no differences when we compare our specimens, descriptions and color photographs with our European specimens, descriptions and colored reproductions. Not only is this the first member of the Luridi found in Alaska to our knowledge, but also it is the first time that we have been certain of *B. erythropus* in North America. We now suspect, however, that some of Paul Marshall Rea's water-color drawings of a California bolete represent *B. erythropus* (cf. **12**, p. 380).

Some luridaceous boleti collected at Bridgewater, Vermont, in 1932, were puzzling at the time and have since been without a definite name, only doubtfully as *B. erythropus*. In the most recent and more complete studies of the European Luridi, we have become convinced that these Vermont specimens are *Boletus Queletii* Schulzer. In fact, if our water-color drawings were added to those of Kallenbach's plate 8 (**3**, Lieferung 4, as *B. erythropus* Pers.), one would not be able to distinguish ours from his. The single difference of any sort is the size of the spores. Those of *B. Queletii* have a quotient of length/breadth of 2. In our Vermont specimens, $Q = 2$ only in the smaller spores and a minority of the others.

BOLETELLUS PROJECTELLUS

In 1937, L. O. Overholts sent a bolete he collected in central Pennsylvania which puzzled him. It had the coloration and spores of *Boletellus mirabilis* (Murrill) Singer but was not as stocky as most western specimens of that species, was not as conspicuously adorned on the

pileus, and besides was "a long way from home" (at least 1200 miles from its most eastern known occurrence in Manitoba). Overholts asked if this collection could be *B. mirabilis*. The one of us who perhaps should have known better assured him that he was correct and, in spite of Singer's expression of surprise at the Pennsylvania extension of area (7, p. 129), over the years one of us has stubbornly clung to his original statement.

Recently, however, in a study of some other specimens, it became necessary to obtain a proper understanding of *B. projectellus* (Murrill) Murrill and we soon brought out Overholts's collection from Pennsylvania. The largest spores of this reach a limit of $28 \times 10.3\ \mu$ and are thus somewhat smaller than the extreme range of 30 or $32 \times 11\text{–}12\ \mu$ given by Singer for *B. projectellus*, but somewhat larger than the largest —$26 \times 9\ \mu$—given by Coker and Beers. The colors and other characters are those of *B. projectellus*, especially the lack of the conic papillations characteristic of *B. mirabilis*, and the host association is with pines rather than fir, hemlock and western red cedar. Consequently, we are now certain that Overholts's collection is not *B. mirabilis* so far out of its normal range, but *B. projectellus* somewhat farther north than Virginia and North Carolina, where it has heretofore been known.

In connection with the foregoing, we were soon reminded of a 1937 specimen from Round Lake (near Saratoga Springs), N. Y., which had been put aside with questions. It was found near pines and hardwoods, and in superficial appearance is indistinguishable from *B. projectellus*. The spores, however, while broad enough ($5.5\text{–}7\ \mu$), are only $14\text{–}17\ \mu$ long. The only other species we know with spores of this size are in the genus *Leccinum* and the very uncertain *Boletus dichrous* Ellis. Any *Leccinum* must be dismissed from consideration—even the similarly colored *L. rubropunctum* (Peck) Singer—because species of this genus do not have a reticulate stipe, and *B. dichrous* not only does not have a reticulate stipe, but also turns blue in the flesh and hymenophore. Therefore, the best that we can do at present is to hold our Round Lake specimen as a questionable small-spored *Boletellus projectellus*, quite far north of what appears to be its normal range.

A NEW SPECIES

This was found originally in Connecticut just over the Rhode Island line a few years ago and was collected again in Pennsylvania in 1960.

Xanthoconium purpureum Snell & Dick, sp. nov.

Pileo impolito vel sublucenti, glabro, "Ox-blood Red" vel "Maroon," 3–7 cm lato. Carne cremeo-albida. Hymenophoro adnato, primo albido, deinde ochraceo

vel ferrugineo-ochraceo, poris parvis. Stipite deorsum plus minusve dilatato, minutissime subpruinoso vel glabro, apice substriato, basi albotomentoso, pallide flavido vel brunneoflavido, plus minusve rubrotincto et rubrovirgato, 5–8 cm × 7–15 mm. Sporis in cumulo paene "Yellow Ochre" vel "Antimony Yellow," oblongo-ellipsoideis vel subfusiformibus, (7–)9–13(–14) × 3–4 μ.

In quercetis. Connecticut et Pennsylvania, U. S. A.

Pileus 3–7 cm broad. Surface dull to dull-shiny, glabrous, rarely with an occasional fibrillose patch, Ox-blood Red to Maroon. Flesh creamy-whitish. Hymenophore adnate, at first whitish, becoming ochraceous or ferruginous-ochraceous, pores small. Stipe more enlarged downward, more or less striate at the apex, very minutely subpruinose to glabrous, more or less white-tomentose at very base; pallid yellowish to brownish-yellowish, tinged reddish in places especially above, more or less reddish-streaked especially at the apex; within creamy-whitish, dingy or brownish below; 5–8 cm long, 7–15 mm thick. Spores in deposit about Yellow Ochre or slightly more ferruginous where thick, about Antimony Yellow where thin, oblong-ellipsoid to subfusiform, pale yellowish, (7–)9–13(–14) × 3–4 μ, mostly 10–11 × 3.5 μ.

Under oaks. Connecticut and Pennsylvania.

TYPE—in WHS Bolete Herbarium, no. 2193.

NEW COMBINATIONS

Singer suggested (**8**, p. 257 and 278) that three species placed in the genus *Boletinus* should be in *Suillus*. These changes are made as follows:

Suillus glandulosus (Peck) Snell & Dick, comb. nov. (basionym: *Boletinus glandulosus* Peck, Bull. N. Y. State Mus. **131**: 34. 1909), tentatively at least in Singer's Subsection Latiporini of the Section Granulati.

Suillus flavoluteus (Snell in Snell & Dick) Snell & Dick, comb. nov. (basionym: *Boletinus flavoluteus* Snell in Snell & Dick, Mycologia **33**: 34. 1941), in Singer's Subsection Hirtellini of the Section Granulati.

Suillus punctatipes (Snell & Dick) Snell & Dick, comb. nov. (basionym: *Boletinus punctatipes* Snell & Dick, Mycologia **33**: 36–37. 1941), in Singer's Subsection Latiporini of the Section Granulati.

SUMMARY

A description is given for one new species, *Xanthoconium purpureum*. The following four new combinations are made: *Xerocomus*

alutaceus, Suillus glandulosus, Suillus flavoluteus, and *Suillus punctatipes. Suillus albidipes* is reduced to a subspecies of *S. granulatus.*

Suillus granulatus subspecies *Snellii* and *albidipes,* and *S. placidus* are differentiated.

Notes are given concerning various aspects of the following species: *Boletus glabellus, B. calopus, B. separans, B. erythropus, B. Queletii, Suillus sibiricus, Boletellus projectellus,* and a species that is possibly *Boletus junquilleus.*

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A SIMPLE TECHNIQUE FOR INVESTIGATING STROMATAL FORMATION IN THE SCLEROTINIACEAE

CARMINE NOVIELLO AND RICHARD P. KORF[1]

(WITH 12 FIGURES)

The stromata of the Sclerotiniaceae, whether of the sclerotial or substratal types, have become important in an understanding of the relationships among the genera of this family, as Whetzel (**1945**) has pointed out. Natural substrata have, for the most part, been used for the study of these stromata by various workers, but these offer one prime difficulty in comparisons of different genera or even different species of the same genus: a critical comparison is impossible when both fungi are unable to colonize the same natural substratum. The following technique was devised in order to overcome this difficulty, and to provide the answers to other, related problems.

MATERIALS

Eight genera of the Sclerotiniaceae of various stromatal types (and two unconnected species of *Botrytis*) were selected for preliminary work:

Botryotinia calthae Whetzel ined. (G. L. Hennebert *2059*)
B. fuckeliana (de Bary) Whetzel (Mary Elliott *B162*)
B. globosa Buchwald (G. L. Hennebert *1704-1*)
B. pelargonii Roed (G. L. Hennebert *1152*)
B. squamosa Viennot-Bourgin (Walker/J. Lorbeer)
Botrytis tulipae (Lib.) Lind (G. L. Hennebert *428*)
Botrytis of the *cinerea*-type from *Ficus* (Noviello, CUP 45254)

[1] The authors wish to express their appreciation to Dr. G. L. Hennebert and Miss Mary E. Elliott, Plant Research Institute, Ottawa; Prof. Thomas Sproston, Jr., University of Vermont; and Prof. James Lorbeer, Cornell University, for transfers of some of the fungi utilized. Mr. Howard Lyon, Cornell University, is responsible for the photography.

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Ciborinia erythronii (Whetzel) Whetzel (L. R. Batra)
Lambertella copticola Korf ined. (Korf/T. Sproston)
L. hicoriae Whetzel nom. subnud. (Korf/T. Sproston)
Myriosclerotinia sulcata (Whetzel) Buchwald (Korf *2997*)
Rutstroemia paludosa (Cash & Davids. *in* Davids. & Cash) Groves & Elliott (Noviello & Korf)
Rutstroemia sp. (Mary Elliott *705*)
Sclerotinia asari Whetzel ined. (CUP 45392)
S. sclerotiorum (Lib.) de Bary (G. L. Hennebert *129*)
S. trifoliorum Eriksson (Buchwald *1275*/G. L. Hennebert *832*)
Seaverinia geranii (Seaver & Horne) Whetzel (G. L. Hennebert)
Streptotinia arisaemae Whetzel (CBS/G. L. Hennebert *1130*)
Streptotinia sp. on *Caulophyllum* (G. L. Hennebert *2047*)

Potato-dextrose broth was made from 200 g peeled potatoes steamed for one hour in 500 ml water, filtered through gauze and brought back to volume with water, to which was added 500 ml water containing 20 g dextrose. The resultant mixture was autoclaved for 20 minutes at 15 lb pressure in 50 ml aliquots in 125 ml Erlenmeyer flasks. For certain experiments 20 g of Difco agar per liter of potato-dextrose broth was added.

Cotton employed was Johnson & Johnson, "Lee" brand, nonsterile, absorbent cotton, obtained in 1 lb rolls.

THE TECHNIQUE

Discs of cotton, *slightly smaller* in diameter than the inside diameter of the 9 cm petri dishes employed, were cut and placed in petri dishes. The thickness of these discs was that of the cotton as provided in the commercially packed roll (about 7 mm). The petri dishes so prepared were placed in groups of six in small paper bags and autoclaved for 30 minutes at 15 lb pressure. (Cotton sterilized in petri dishes with dry heat did not absorb the medium as quickly in our experience.)

Approximately 50 ml of sterile potato-dextrose broth was added aseptically to each prepared sterile petri dish with its cotton disc (FIG. 1). The amount of broth depends upon the thickness of the disc, for the desired result is absorption of the medium without excess free liquid.

A small disc of mycelium from the growing margin of a colony on potato-dextrose agar was placed on the upper surface in the center of the cotton disc, and the plates were then placed in incubators at 15, 18, 21 and 24° C. Typical stromata developed under these conditions (except in the case of *Seaverinia geranii*, on which further studies are

in progress), though at different rates for each species and each temperature (FIGS. 2–7). Control cultures run simultaneously on potato-dextrose agar gave essentially identical results, except that the stromata on agar were frequently noted to be fewer in number and smaller in size than those produced on the cotton discs.

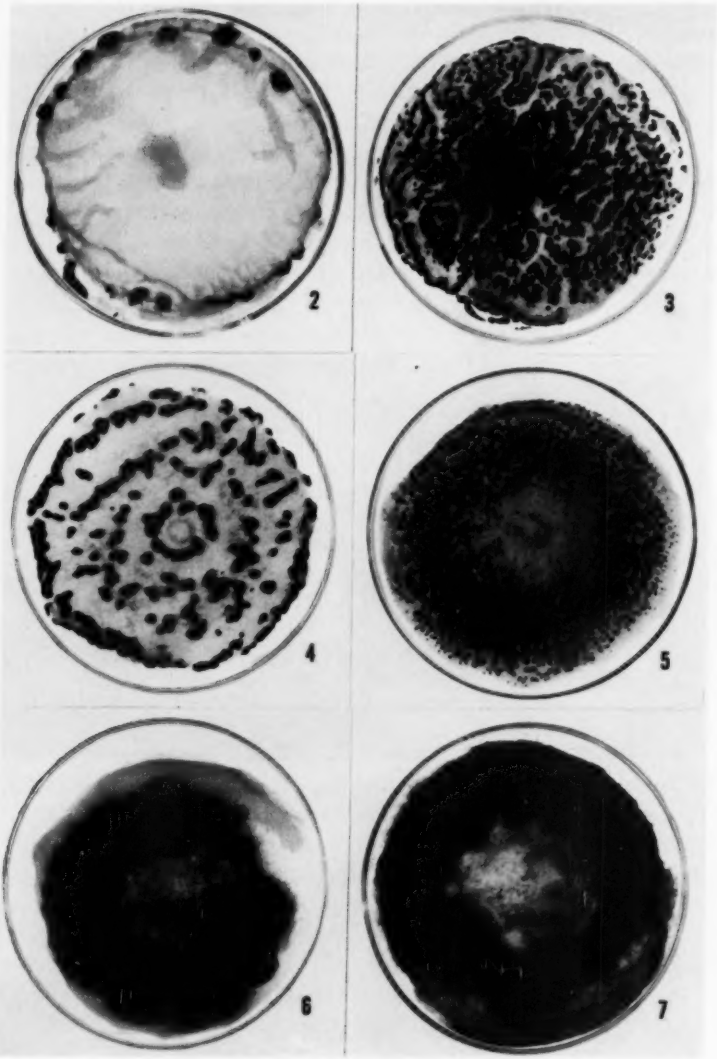
A variant of the technique was used in early experiments, in which a basal layer of potato-dextrose agar was allowed to solidify in a sterile petri dish, then seeded with the fungus. Over this was placed a wet disc of cotton which had been sterilized in distilled water. Very similar results were obtained, but they are not wholly comparable, since, for example, it was noted that in *Ciborinia erythronii*, the stromata were

FIG. 1. Pouring potato-dextrose broth into a petri dish containing a sterilized cotton disc.

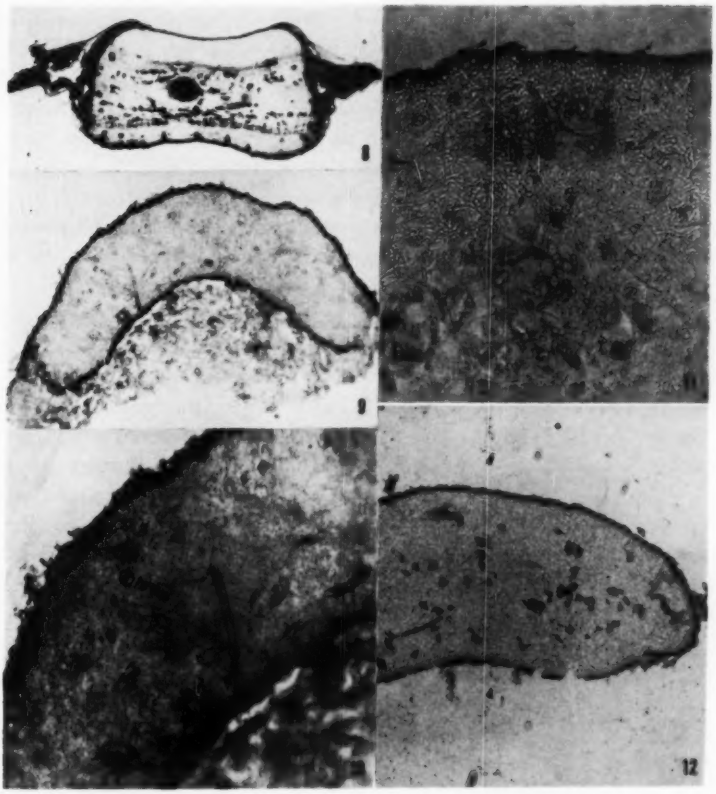
scattered when an agar base was used, and more gregarious when the broth was used. The agar technique was abandoned, however, since there is a much greater chance of introducing contaminants at the time of transfer of the wet cotton disc.

PRELIMINARY OBSERVATIONS

There remain many fundamental questions to be answered in regard to stromatal formation in the Sclerotiniaceae. One important question pertains to the significance to be attached in classification to the mode of development of the stromata and their relationship to the substratum. Whetzel (**1945**) was clear to point out, for example, that the tuberoid



FIGS. 2–7. Cultures of Sclerotiniaceae on cotton discs, 15 days old. Somewhat reduced; 2. *Sclerotinia sclerotiorum*, 21° C; 3. *Ciborinia erythronii*, 24° C; 4. *Botryotinia pelargonii*, 18° C; 5. *Streptotinia arisaemae*, 15° C; 6. *Lambertella hicoriae*, 21° C; 7. *Rutstroemia paludosa*, 24° C.



FIGS. 8–12. Comparison of stromata developed in leaves and in cotton discs. All material sectioned on a freezing microtome; 8–10. *Botryotinia fuckeliana;* 8. Young stroma developing within a leaf of *Ficus clastica* cv. Decora; 9. Young stroma in cotton disc; 10. Enlargement of portion of FIG. 9, showing cotton fibers embedded in the medulla; 11–12. *Ciborinia erythronii;* 11. Mature stroma developed in the leaf of *Erythronium americanum,* showing (at arrow) embedded remnants of vascular elements; 12. Mature stroma developed in cotton disc showing embedded cotton fibers.

sclerotia of *Sclerotinia* are formed free on the mycelium, and are devoid of remnants of suscept tissue, while the discoid sclerotium of *Ciborinia* is characterized by the inclusion of undigested vascular elements and other host debris within the stroma. Similar development to that in *Ciborinia* was noted by the senior author (Noviello, **1962**) in a *Botrytis* of the *cinerea*-type attacking *Ficus clastica* cv. Decora, and in *Ovulinia*

azaleae Weiss by Hemmi and Akai (**1959**), for example. The technique seems to provide an answer, at least for the species which we have investigated so far, since all three species of *Sclerotinia* used develop sclerotia which are free from the cotton disc at maturity, and have no cotton fibers inside. On the other hand, cotton fibers are abundant in the medulla of the sclerotia of all species of *Botryotinia, Botrytis, Ciborinia* and *Streptotinia* used. The development of the stromata in the cotton discs appears to be essentially the same as within the leaf in the case at least of *Botryotinia fuckeliana* and *Ciborinia erythronii*, the only two species in which an experimental comparison has been made by the authors. Because of the senior author's earlier work on the *Botrytis* disease of *Ficus*, this suscept was chosen for inoculation with a known strain of *Botryotinia fuckeliana* (stat. conid. *Botrytis cinerea*). As reported for the *Botrytis* earlier (Noviello, **1962**), *Botryotinia fuckeliana* forms its sclerotia within the *Ficus* leaf (FIG. 8), leaving undigested portions of the leaf embedded in the medulla, clearly outlining the multiple epidermis, the palisade layer, and the stomatal chambers of the lower epidermis. On cotton discs (FIG. 9) the sclerotia are simpler in outline, but contain in the medulla many fragments of undigested cotton fibers (FIG. 10). A wholly similar phenomenon is exhibited by *Ciborinia erythronii*, which when it forms its sclerotia in the leaves of *Erythronium americanum* shows remnants of vascular elements embedded in the medulla (FIG. 11), while when grown on cotton discs shows fragments of cotton dispersed throughout the medulla (FIG. 12).

A second question which has puzzled us has been the nature of—and the presence or absence of—a "flexible to gelatinous matrix" in which the hyphae of the medulla of some genera are embedded (Whetzel, **1945**). This matrix is clearly absent in *Sclerotinia*, for example, and evident in *Botryotinia*. Our early studies of *Ciborinia* led us to believe that a matrix embedding the hyphae was present, despite Whetzel's (**1945**) statement to the contrary. This technique has allowed us to examine sclerotia which have developed free from any agar, and likewise free from the leaf proteins which might form a confusing gel. We are now convinced that a matrix—of as yet unknown nature—is certainly present in *C. erythronii*. This lends additional strength to our earlier contention (Noviello, **1962**) that *Botryotinia* and *Ciborinia* may prove to be more closely related to each other than is either to *Sclerotinia* sensu stricto.

We are convinced that this technique will prove of value in elucidating also the relationships of other genera, and may answer some of the still perplexing problems concerning the true nature of the substratal

stromata and the sclerotia found in the various genera of the Sclerotiniaceae.

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HETEROCARYOSIS AND SOMATIC RECOMBINATION IN CEPHALOSPORIUM MYCOPHILUM [1]

R. W. Tuveson and D. O. Coy

INTRODUCTION

Heterocaryosis may be defined as the association of genetically dissimilar nuclei in a single cytoplasmic unit. Previous investigations with *Fusarium* (Tuveson and Garber, **1959b** and **1961**; Coy and Tuveson, **1961**) have shown that certain heterocaryons yield conidia with nuclei carrying markers characteristic of only one of the component strains. These results were interpreted by assuming that selective forces favored the multiplication of one nuclear type or the incorporation of one nuclear type in the conidia. Further, these presumptive selective forces have been nullified in certain specific heterocaryons of *Fusarium* when these heterocaryons were grown on minimal medium supplemented with the nutrilites for the synthesis of which the "major" nuclear component was responsible. This paper presents evidence for nonselective and selective incorporation of nuclear types in the conidia of heterocaryons between auxotrophic strains of *Cephalosporium mycophilum* (Cda.) Tubaki strain SJ–104 when grown on minimal medium. In addition, evidence is presented for the alteration of nuclear proportions when heterocaryons of *C. mycophilum* are grown on supplemented minimal medium.

This paper also presents evidence for somatic recombination of genetic factors in *C. mycophilum*, a fungal species which does not exhibit a well-defined sexual cycle.

MATERIALS AND METHODS

The stock of *Cephalosporium mycophilum* was supplied by Dr. M. Pisano of St. John's University, Jamaica, New York. The strain was originally isolated from soil and identified at the Centraalbureau voor Schimmelcultures, Baarn, Netherlands.

The complete medium employed throughout these investigations was potato dextrose agar supplemented with 0.5% yeast extract (Difco).

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The minimal medium used in all experiments was Czapek's solution agar (Difco).

Methods for the induction, isolation and characterization of nutritional mutants were identical to those used in previous investigations with *Fusarium* and have been described (Tuveson and Garber, **1959a**).

For the synthesis of heterocaryons, conidia from the auxotrophic strains were harvested from actively growing cultures by pouring 9 ml of sterile distilled water over the colony and agitating with sterile glass beads to loosen the conidia. A hemocytometer count of the resulting suspension was made and the concentration of spores was adjusted to equality before mixing. No viable counts were made. Before mixing, each mutant was plated on minimal medium to check for reversion. In addition, each combination of auxotrophs was checked for cross feeding by parallel streaking on minimal agar plates. The auxotrophic conidial suspensions were mixed and added to the surface of a complete medium plate which had been overlaid with 9 ml of sterile distilled water. The mycelial mat which resulted after 4–7 days of incubation was removed and washed in four changes of sterile distilled water. The mat was shredded and the fragments were added to the surface of minimal agar.

The mycelial fans which appeared at the periphery of the fragments after a week of incubation were transferred to minimal agar plates, and their identification as heterocaryons was validated by continued growth after transfer.

All cultures were grown in an incubation room in which the temperature varied between 20 and 26 degrees C.

The nutritional requirements of conidia harvested from the presumptive heterocaryons were determined by replicate plating techniques as described by Coy and Tuveson (**1961**).

Conidia harvested from the presumptive diploid colonies grown on complete medium were diluted and plated on complete medium producing colonies derived from single conidia. These colonies were selected randomly and positioned on grids of twenty-six colonies for replicate plating on plates containing three of the four (or four of the five) nutrilites required by the parental auxotrophic strains to determine their nutritional requirements.

With the exception of cysteine which was added to the minimal medium at the rate of .4 mg/ml of the medium, the concentrations of the nutrilites employed as supplements in the minimal medium have been presented (Tuveson and Garber, **1959b**).

The nutritional requirements and colors of the auxotrophic strains employed in this investigation were: -met, gr (-methionine, green);

-arg, gr (-arginine, green); -ade, or (-adenine, orange); -met, wh (-methionine, white); -ade, -met, or (-adenine, -methionine, orange); -arg, -leu, -cys, gr (-arginine, -leucine, -cysteine, green).

RESULTS

In preliminary experiments, a heterocaryon was formed between two monoauxotrophic strains of *Cephalosporium mycophilum*. The conidia from this heterocaryon (HET I–C) growing on minimal medium were harvested and their nutritional requirements determined. The data from this experiment are presented in TABLE I. When HET I–C was grown

TABLE I

NUTRITIONAL REQUIREMENTS OF CONIDIA HARVESTED FROM HET I–C (COMPONENT STRAINS -MET, GR + -ARG, GR)

Medium on which heterocaryon was grown		Supplements added to minimal medium for identification of nutritional requirements of conidia			
		minimal control	methionine + arginine	methionine	arginine
minimal	$\bar{x}\pm$s.e.*	0	109 ±4.0	81.5±2.8	18.7±1.1
		minimal control	methionine + arginine	methionine	arginine
minimal + .4 mg/ml methionine	$\bar{x}\pm$s.e.	0	175.9±6.3	161.6±7.2	0.4**
		minimal control	methionine + arginine	methionine	arginine
minimal + .4 mg/ml arginine	$\bar{x}\pm$s.e.	0	111.2±2.6	4.7±1.1	138.6±5.1

* mean of ten replicates plus or minus the standard error.
** mean of ten replicates.

on minimal medium, both component nuclear types were recovered as evidenced by the appearance of colonies on plates supplemented with methionine alone and on plates supplemented with arginine alone.

HET I–C was grown on minimal medium supplemented with methionine and on minimal medium supplemented with arginine. The conidia were harvested from these colonies grown on supplemented minimal medium and their nutritional requirements determined. The data from these experiments is also presented in TABLE I. When HET I–C was grown on minimal medium supplemented with methionine, a preponderance of methionine deficient nuclei appeared in the conidia produced by the heterocaryon. Conversely, when HET I–C was grown on minimal medium supplemented with arginine, a preponderance of arginine deficient nuclei was recovered.

A second heterocaryon (HET II–C) involving monoauxotrophic strains of *C. mycophilum* was synthesized, grown on minimal medium and the nutritional requirements of the resulting conidia analyzed. The results of this experiment are presented in TABLE II. When grown on minimal medium, HET II–C yielded conidia bearing markers representative of only one of the component strains (-ade, or). The methionine deficient component was not detected. HET II–C was grown on minimal medium supplemented with methionine and the nutritional requirements of the conidia determined (TABLE II). When grown on minimal medium supplemented with methionine, HET II–C yielded conidia bearing nuclei which were deficient for the synthesis of methionine. The adenine deficient component was not observed.

TABLE II

NUTRITIONAL REQUIREMENTS OF CONIDIA HARVESTED FROM HET II–C (COMPONENT STRAINS –MET, WH + –ADE, OR)

Medium on which heterocaryon was grown	Supplements added to minimal medium for identification of nutritional requirements of conidia			
	minimal control	methionine + adenine	methionine	adenine
minimal $\bar{x}\pm$s.e.*	0	69.9±2.8	0	74.1±3.2
	minimal control	methionine + adenine	methionine	adenine
minimal + .4 mg/ml methionine $\bar{x}\pm$s.e.	0	115.0±4.5	107.4±4.1	0

* mean of ten replicates plus or minus the standard error.

A third heterocaryon (HET III–C) was synthesized involving a monoauxotrophic strain (-ade, or) and a triauxotrophic strain (-arg, -leu, -cys, gr). The nutritional requirements of the conidia harvested from this heterocaryon grown on minimal medium are presented in TABLE III. Again, as had been observed in HET II–C, the adenine deficient component was recovered exclusively when the heterocaryon was grown on minimal medium. When HET III–C was grown on minimal medium supplemented with arginine, leucine and cysteine for a period of three weeks and the nutritional requirements of the conidia determined, both nuclear components were recovered with, however, the triauxotrophic component occurring with the greater frequency (TABLE III).

When HET III–C was growing in minimal medium, a fan of rapidly growing mycelium emerged from one of the heterocaryotic colonies. This fan was removed to another minimal plate and incubated for two

weeks. The heterocaryotic colony from which the fan emerged was orange, but the transfer from the rapidly growing fan produced a white colony which formed sectors of several colors (orange, green and orange-green) as it developed. The appearance of colored sectors suggested that this colony might be a somatic diploid which was undergoing somatic recombination for visual markers (color). It is hypothesized that the orange and green colors of the parental strains are the result of mutations at different loci. The white color of the somatic diploid (DIP I–C) is presumed to be the result of dominant, complementary alleles carried by the parental strains. The sectoring, white presumptive diploid was transferred to complete medium in order that selection against possible auxotrophic recombinant sectors would not occur. The

TABLE III

NUTRITIONAL REQUIREMENTS OF CONIDIA HARVESTED FROM HET III–C (COMPONENT STRAINS −ADE, OR + −ARG, −LEU, −CYS, GR)

Medium on which heterocaryon was grown		Supplements added to minimal medium for identification of nutritional requirements of conidia				
	minimal control	ade, cys,** leu, arg	ade, cys, leu	ade, cys, arg	ade, leu, arg	cys, leu, arg
minimal $\bar{x}$±s.e.*	0	86.9± 2.8	84.3± 2.6	85.2± 3.0	85.8± 2.0	0
	minimal control	ade, cys, leu, arg	ade, cys, leu	ade, cys, arg	ade, leu, arg	cys, leu, arg
minimal + .4mg/ml arginine + .8 mg/ml leucine + .4 mg/ml cysteine $\bar{x}$±s.e.	0	121.1± 5.6	10.1± 0.9	10.6± 0.9	8.6± 1.3	128.2± 3.7

* mean of ten replicates plus or minus the standard error.
** ade, adenine; cys, cysteine; leu, leucine; arg, arginine.

presumptive diploid was allowed to develop for three weeks on complete medium at the end of which time conidia were harvested, plated on complete medium and the total phenotype of 361 randomly selected colonies was determined. The data from this experiment are presented in TABLE IV. It is possible that the monoauxotrophic colonies are the product of somatic crossing over resulting in homozygosis for certain alleles carried in the heterozygous condition in the original somatic diploid or that they may represent products of haploidization bearing single auxotrophic markers. The multiauxotrophs may represent the products of haploidization resulting in several markers being unmasked. The markers are, for the present, assumed to be independent, for the frequencies with which colonies of a particular phenotype were detected cannot be

assumed to be significant and indicative of linkage since they may simply represent clones. This would be possible if certain recombinational events had occurred early in the development of the diploid colony resulting in unusually large sectors which would be represented disproportionately the total conidial population. Even when it is assumed that identical genotypes represent clones, 5.7% of the conidia analyzed

TABLE IV

PHENOTYPES OF REPRESENTATIVE AUXOTROPHIC CONIDIA PRODUCED BY SOMATIC DIP I–C (PARENTAL STRAINS –ADE, OR & –ARG, –LEU, –CYS, GR)

Presumed Genotype of White Somatic Diploid

$\frac{+}{g}$ $\frac{o}{+}$ $\frac{ade}{+}$ $\frac{+}{cys}$ $\frac{+}{leu}$ $\frac{+}{arg}$

Monoauxotrophs	Frequency of colonies exhibiting phenotype
-leucine, white	3
-cysteine, white	18
-arginine, white	3
-adenine, white	10
-cysteine, orange	14
-arginine, orange	3
-adenine, orange (parental)	6
-cysteine, green	3
-leucine, green	1
-adenine, green	7
-cysteine, orange-green	2
-adenine, orange-green	3
Multiauxotrophs	
-leucine, -arginine, -cysteine, white	1
-leucine, -adenine, -cysteine, white	1
-arginine, -leucine, orange	2
-cysteine, -leucine, -adenine, orange	1
-arginine, -leucine, green	1
-leucine, -adenine, orange-green	1

361 total colonies analyzed
auxotrophs = 21.9%
distinct combinations = 6.0% (assuming clones)
recombinants = 5.7% (assuming clones)

represent recombinants. Several of the genotypes represented in TABLE IV are clearly examples of recombination between the two independently derived parental strains. For instance, the -cysteine, -leucine, -adenine, orange triauxotroph carries two alleles from each of the respective parental strains.

A second somatic diploid (DIP II–C) was isolated and carried the same alleles as DIP I–C with the exception of an additional marker in

the monoauxotrophic parent (-ade, or) giving an -adenine, -methionine, orange diauxotroph. As was the case with DIP I–C, DIP II–C yielded a white colony which produced colored sectors. Unlike DIP I–C, the sectors in this instance were all orange with green and orange-green not being observed. The total phenotype of a random sample of conidia from DIP II–C was determined and the data are presented in TABLE V. Again, the frequency with which individual phenotypes appear cannot be considered significant for clones may be represented. If we assume

TABLE V

PHENOTYPES OF REPRESENTATIVE AUXOTROPHIC CONIDIA PRODUCED BY SOMATIC DIP II–C COLONY (PARENTAL STRAINS –ADE, –MET, OR & –ARG, –LEU, –CYS, GR)

Presumed Genotype of White Somatic Diploid						
+ / g	o / +	ade / +	met / +	+ / cys	+ / leu	+ / arg

Monoauxotrophs	Frequency of colonies exhibiting phenotype
-cysteine, white	62
-adenine, white	8
-methionine, white	5
-cysteine, orange	44
-adenine, orange	4
-methionine, orange	9
-leucine, orange	1
Multiauxotrophs	
-adenine, -methionine, white	1
-adenine, -cysteine, white	7
-adenine, -methionine, orange (parental)	4
-adenine, -cysteine, orange	26
-adenine, -leucine, -methionine, orange	1

289 total colonies analyzed
auxotrophs = 59.5%
distinct combinations = 9.3% (assuming clones)
recombinants = 8.5% (assuming clones)

that the frequencies do represent clones, 9.3% of the conidia analyzed represent distinct combinations of phenotypes and 8.5% are recombinants. Again recombination between the two parental strains is evidenced as in the -adenine, -leucine, -methionine, orange triauxotroph which bears markers representative of the two parental strains. It should be noted that although the allele which determines green color was not detected, the nutritional markers associated with green in the parental strain did appear among the recombinants.

DISCUSSION

The heterocaryons studied within *Cephalosporium mycophilum* when grown on minimal medium may or may not exhibit selection for the incorporation of a single nuclear type in the conidia. In HET I–C, neither nuclear component was selected on minimal medium for incorporation into the conidia. Selection for the incorporation of one of the nuclear components in the conidia was observed in HET II–C and HET III–C when these heterocaryons were grown on minimal medium It is interesting to note that in HET II–C and HET III–C the nuclear component common to both heterocaryons (-ade, or) was the one recovered in the conidia from these heterocaryons. The significance of this observation cannot be evaluated from the present data.

In the three heterocaryons investigated, it was possible to alter the nuclear proportions of heterocaryons grown on minimal medium by supplementing the minimal medium with specific nutrilites. The nuclear proportions in heterocaryons of *C. mycophilum* are not rigidly determined but are characteristic of the medium on which the heterocaryon develops. This is in contrast with the situation in *Neurospora* (Pittenger and Atwood, **1956**) in which it was found that nuclear proportions are relatively constant during growth and any slight changes that do occur are not adaptive. Further, it was found that in the *Neurospora* heterocaryons investigated supplementation did not alter nuclear proportions. Ryan and Lederberg (**1946**) have reported that when a *Neurospora* heterocaryon involving leucine independent and leucine deficient nuclei was grown on a medium supplemented with leucine only leucine nuclei were recovered. Jinks (**1952**) in working with heterocaryons between morphological mutants of *Penicillium cyclopium* found that nuclear proportions were characteristic of the medium on which the heterocaryon was grown. Buxton (**1954**) has reported similar observations for heterocaryons between two morphological mutants of *Fusarium oxysporum* f. *gladioli*. Certain specific heterocaryons between nutritionally deficient strains of *Fusarium oxysporum* f. *pisi* (Tuveson and Garber, 1961), *F. solani* f. *cucurbitae* and between the two species (Coy and Tuveson, 1961) have been shown to alter their nuclear proportions when grown on supplemented minimal medium.

In the three heterocaryons investigated in *C. mycophilum* in which an attempt was made to induce alterations in nuclear proportions by supplementation of the minimal medium, each heterocaryon responded by altered nuclear proportions. In *Fusarium* (Tuveson and Garber, **1961**; Coy and Tuveson, **1961**) only selected heterocaryons responded to supplementation of the medium by altered nuclear proportions. It is possi-

ble that when other heterocaryons within *C. mycophilum* are investigated that specific ones may not respond to supplementation by altering nuclear proportions.

Our results indicate that *C. mycophilum* is able to recombine genetic factors although no sexual stage is known for this species. Our preliminary results indicate that the method of recombination may be analogous to that observed by Pontecorvo and Käfer (**1958**) in *Penicillium* and *Aspergillus*. The events leading to recombination appear to occur rather frequently since the proportion of recombinants among the spores examined was 5.7% and 8.5% for the respective diploids. These may be underestimates since identical genotypes were assumed to be clonal for these calculations.

The failure to detect the green marker in recombinants from DIP II–C might simply represent an earlier haploidization for this marker, or homozygosis of the locus through crossing over, before isolation of the diploid.

These results suggest that a genetic analysis of *Cephalosporium mycophilum* may be accomplished somatically. In addition, our results suggest the possible occurrence of somatic recombination in other members of this genus some of which are important plant pathogens and antibiotic producers.

SUMMARY

Three heterocaryons between auxotrophic strains of *Cephalosporium mycophilum* have been investigated. In two of the heterocaryons grown on minimal medium, only one of the component nuclear types could be detected in the conidia.

Each of the three heterocaryons investigated responded to supplementation of the minimal medium on which they were grown by alteration of nuclear proportions.

Analyses of conidia produced by two presumptive somatic diploid colonies have yielded recombinant nuclei bearing markers characteristic of the two parental strains. These investigations suggest the possibility of carrying out genetic analyses in *C. mycophilum*, a fungus lacking a well defined sexual cycle.

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A SPECIES OF ENDOGONE FROM CORN CAUSING VESICULAR–ARBUSCULAR MYCORRHIZA

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(WITH 11 FIGURES)

Phycomycetous, or vesicular-arbuscular, mycorrhizas are common on a wide variety of wild and cultivated plants. Until recently relatively little was known of the systematic position of the fungi involved. Peyronel (**12**, **13**, **14**) was able to trace hyphal connections between mycorrhizal roots and sporocarps of three species of *Endogone*. Butler (**1**) noted that the vesicles produced on hyphae attached to mycorrhizal roots resembled the chlamydospores of the Endogonaceae. Dowding (**2**) found chlamydospores and fruiting bodies of *Endogone fasciculata* Thaxter associated with mycorrhizal roots of plants growing in Alberta swamps. Nicolson (**11**) reported external vesicles, and vesicular aggregates associated with vesicular-arbuscular mycorrhizal infections in species of Gramineae. The vesicular aggregates resembled fruiting bodies of certain species of *Endogone*. Mosse (**9**) found fructifications of an undescribed species of *Endogone* attached to mycorrhizal strawberry roots. Typical vesicular-arbuscular mycorrhizal infections were produced in strawberry, apple, wheat, grasses, tomato, lettuce, onion and barley roots inoculated with sporocarps from strawberries. Positive evidence of the relationship of the *Endogone* species and mycorrhizal infection was obtained by inoculating strawberry seedlings with individual surface sterilized spores in plugged test tubes containing autoclaved soil. The infections obtained from inoculations were similar in every respect to those occurring in nature. Koch (**7**) and Meloh (**8**) studied a species of *Endogone* that produced endotrophic mycorrhizal infections. This species produced arbuscules; however, vesicles were not observed. Gerdemann (**3**), by wet sieving and decanting, obtained large yellow spores (B spores) from Illinois soils. These spores were one-celled and had a thin outer and a thick inner wall. They resembled spores of the Endogonaceae in their size and general appearance. Phycomycetous mycorrhiza developed in red clover, corn, strawberry and sweet clover roots, inoculated with spores from infected red clover. Arbus-

cules but not vesicles formed in infected roots. Compact clusters of one-celled, clavate, echinulate spores (C spores) were produced on external hyphae.

MACROSCOPIC RECOGNITION OF MYCORRHIZAL CORN

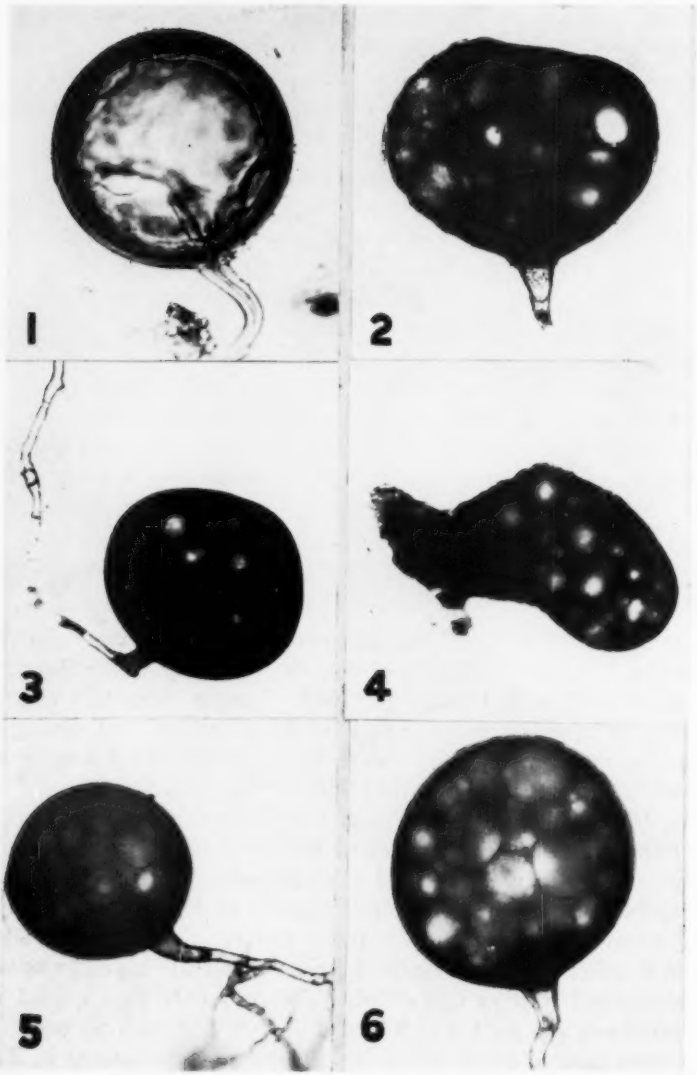
Corn (*Zea mays* L.) roots infected with mycorrhizal fungi are easily recognized macroscopically by their bright yellow color which contrasts sharply with the white appearance of nonmycorrhizal roots. This yellow color which is quite striking on freshly dug roots, rapidly disappears if the roots are exposed to bright light. The association of yellow color with mycorrhizal infection was verified by a microscopic examination of roots from field grown corn. Root samples were collected and divided into two lots on the basis of color. They were then cleared, stained, and examined microscopically. All yellow roots were mycorrhizal and white roots were nonmycorrhizal. This color difference was also reported by Jones (**6**) who noted that mycorrhizal pea roots could be recognized by their "straw yellow" color.

MYCORRHIZAL DEVELOPMENT FROM SOIL FRACTIONS DIFFERING IN PARTICLE SIZE

In the fall of 1959, soil was taken from around the roots of mature corn plants on the University of Illinois Agronomy South Farm, and stored at 5° C until January 28, 1960. Samples from this soil were thoroughly mixed with water and by the process of wet sieving and decanting the lighter particles were separated into the following fractions: 1) larger than 1000 μ, 2) 710–1000 μ, 3) 250–710 μ, 4) 149–250 μ, 5) 105–149 μ, 6) 74–105 μ, and 7) 44–74 μ.

These fractions, which consisted mainly of bits of roots and other organic matter in various stages of decomposition, were added to pots of sterilized soil. The potting soil was a 1 to 3 mixture of sand and soil, steam sterilized in 6 inch pots. Following sterilization, all but about 2 inches of soil was removed from the pots and a small amount of a specific soil fraction was added. The pots were then refilled with sterilized soil, and planted with corn at the rate of 2 seeds per pot. Two pots were planted for each fraction. Corn was also planted in 2 pots of sterilized soil as a check.

After 88 days, the roots were washed free of soil and samples were cleared and stained by boiling for about 10 minutes in a saturated solution of chloral hydrate containing a small amount of acid fuchsin. The roots were then examined in lacto-phenol. Phycomycetous mycorrhizal



FIGS. 1–6. *Endogone*. FIG. 1. Cleared and stained chlamydospore associated with corn roots in pots containing a soil fraction 74 to 105 μ in diameter (fraction 6) (× 270). FIGS. 2, 3, 4. Chlamydospores obtained from field soil by wet sieving and decanting (× 138). FIGS. 5, 6. Chlamydospores produced on hyphae attached to mycorrhizal corn roots inoculated with field collected chlamydospores (× 138).

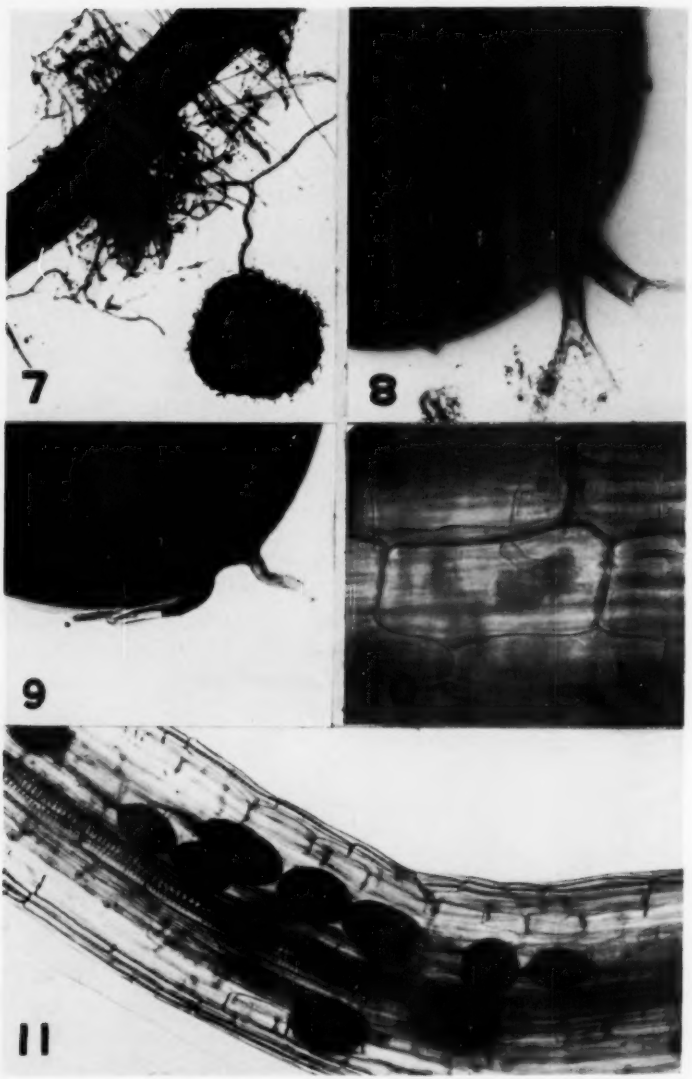
infections were present in roots from all pots to which soil fractions had been added, and both vesicles and arbuscules were common. Several clumps of C spores were found in pots with fractions 3 and 4, indicating that B spores probably were present in these fractions. Sporocarps of an *Endogone* sp. 389 to 830 μ in diameter were abundant on roots in pots containing fraction 4 and a few were also found in pots with fractions 3 and 5. The sporocarps were loosely attached to the roots by hyphae, and many extra-matrical chlamydospores up to 258 μ in diameter were attached to the same hyphae. Smaller thicker-walled chlamydospores (FIG. 1) were common in pots containing fraction 6 and either the same or a similar type of spore was occasionally found in pots containing other size fractions. This type of spore was not associated with sporocarps and apparently it is produced by some other species of *Endogone*.

These results suggest that 3 or more species of phycomycetous mycorrhizal fungi were present in this field soil: 1) a species producing B and C spores; 2) a species of *Endogone* producing sporocarps and extra-matrical chlamydospores; 3) a species producing smaller, thicker walled chlamydospores borne singly, and perhaps a fourth species that produced only vesicles and arbuscules. Since *Endogone* sporocarps and C spores occurred only in pots containing certain fractions, it is probable that these 2 species survived in the soil as spores which fell within the size range of these fractions. It also appears that these species did not survive as mycelium or vesicles in dead roots, since particles of dead roots were common in all fractions.

Inoculation with extra-matrical chlamydospores

The greatest number of sporocarps were produced in pots to which fraction 4 (149–250 μ) had been added. Therefore, the source of inoculum in these pots could not have been sporocarps since their minimum diameters exceed 250 μ.

A 105–710 μ soil fraction from the same collection of field soil was examined under the dissecting microscope. Chlamydospores 133–258 μ in diameter (FIGS. 2, 3, 4) were abundant. These spores appeared identical to those produced in association with *Endogone* sporocarps in pots containing fractions 3, 4 and 5, and their size range suggests that they were present in each of these 3 soil fractions. Several fractions with a size range of 250–1000 μ were examined and after an extensive search 2 sporocarps of *Endogone* were found. Sporocarps were produced abundantly on inoculated plant roots growing in sterilized soil in the green-



FIGS. 7–11. FIG. 7. Sporocarp of *Endogone* attached to a mycorrhizal corn root inoculated with chlamydospores obtained from field soil (× 15). FIGS. 8, 9. Cleared and stained spores from *Endogone* sporocarps showing 2 subtending hyphae (× 270). FIG. 10. Intercellular hyphae and an arbuscule in the cortex of a soybean root inoculated with *Endogone* sporocarps (× 450). FIG. 11. Vesicles in an onion root inoculated with *Endogone* sporocarps (× 138).

house. Under field conditions extra-matrical chlamydospores probably are a more important source of infection.

Extra-matrical chlamydospores were separated from 149–250 μ soil fractions with the aid of a flattened needle and a dissecting microscope. Five pots of steam-sterilized soil were infested by spreading 100 spores over a layer of soil 2 inches deep. The pots were then filled with soil and planted with corn. Soil in one additional pot was left uninfested. The roots were examined after 86 days.

Mycorrhiza developed in all of the infested pots, while roots in the check pots were nonmycorrhizal. Extra-matrical chlamydospores (FIGS. 5, 6) and sporocarps (FIG. 7) were abundant on hyphae attached to mycorrhizal roots. The inoculation experiment was repeated with 50 chlamydospores added per pot. Corn seed was planted in two pots of infested and in one pot of uninfested soil. Only light infection occurred in infested pots. However, a few sporocarps and extra-matrical chlamydospores developed. The unhealthy appearance of the roots suggested that pathogenic fungi had probably been introduced on the chlamydospores. Roots in the check pot were free of mycorrhizal infection.

The sporocarps produced by the Illinois *Endogone* appear similar to those described by Mosse (**9**). They are compact, range in size from 400 to 900 μ in diameter, and contain from 2 to 6 spores 124 to 312 μ in diameter. The endospore is yellow, thick-walled, and the wall extends a short distance into the stalk. The outer wall is thin and is easily separated from the endospore. Many spores have two subtending hyphae (FIGS. 8, 9), suggesting that they may be zygospores. Hawker (**5**) and Godfrey (**4**), after examination of sporocarps obtained from Mosse, interpreted them as chlamydospores. Mosse (**9**) described the sporocarps of her isolate as up to 1 mm in diameter containing as many as 32 (usually 2 to 6) spores, 60–160–250 μ in diameter. The sporocarps of the Illinois isolate tend to have fewer spores of a slightly larger size. The extra-matrical chlamydospores of the Illinois *Endogone* are very similar to the spores produced in sporocarps. They differ in that they never have more than one subtending hypha. Mosse (**10**) described the extra-matrical spores as 20–150 μ in diameter which is considerably smaller than the spores produced by the Illinois *Endogone*. In a species with such a wide range in size of spores, these differences may be of little significance.

Inoculation with sporocarps

Sporocarps that developed on corn roots were used to inoculate corn, red clover, soybean, onion, and strawberry. Mycorrhizal infections oc-

curred on corn roots inoculated with sporocarps. Both arbuscules and vesicles were formed, and extra-matrical chlamydospores and new sporocarps were produced. Vesicular-arbuscular mycorrhiza also developed in roots of red clover, soybean (FIG. 10), onion (FIG. 11), and strawberry inoculated with sporocarps from corn.

SUMMARY

An *Endogone* causing vesicular-arbuscular mycorrhiza has been found on corn in Illinois. The species is similar to the unnamed *Endogone* reported by Mosse from strawberry in England. Extra-matrical chlamyodospores were common in soil collected from around corn roots. Corn plants inoculated with chlamydospores developed typical vesicular-arbuscular mycorrhiza. Sporocarps and extra-matrical chlamydospores were produced on hyphae attached to infected roots. Sporocarps developed abundantly on roots of inoculated plants grown in sterilized soil, but they were rarely found in field soil. The main source of inoculum in field soil appears to be extra-matrical chlamydospores. The fungus also produced vesicular-arbuscular mycorrhiza in roots of strawberry, onion, red clover and soybeans.

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BOTRYODIPLODIA THEOBROMAE AND ITS ASSOCIATION WITH MUSA SPECIES

ROGER D. GOOS, ELSIE A. COX AND G. STOTZKY

(WITH 7 FIGURES)

Botryodiplodia theobromae Pat. is a common and widespread fungus in tropical areas, where it is well-known as a wound parasite on a wide range of host plants. A partial list of the recorded hosts includes citrus (Nowell, **1923**; Pole-Evans, **1910**), rubber (Cook, **1913**; Petch, **1921**), tea (Cook, **1913**; Johnston, **1960**), cacao (Charles, **1906**; Cook, **1913**; Griffon and Maublanc, **1909**; Petch, **1921**; Urquhart, **1961**), sugar cane (Howard, **1901**; Nowell, **1923**), cotton (Johnston, **1960**; Patouillard, **1922**), tung (Large, **1948**), oil palm (Johnston, **1960**; Zambettakis, **1950**), mango (Charles, **1906**; Zambettakis, **1950**), coffee (Massee, **1909**, Riley, **1960**), papaya (Petch, **1921**; Zambettakis, **1950**), castor bean (Riley, **1960**), cashew (Riley, **1960**), tobacco (Averna-Sacca, **1922**), peanut (Wilson, **1947**), and banana (Wardlaw, **1935**). *B. theobromae* is considered by most authors to be a weak pathogen, invading its hosts only through wounds or necrotic tissue. It occurs as a seed-borne fungus in rubber, cotton, and cacao (Noble et al., **1958**), and has also been isolated from soil in Central America (Farrow, **1954**) and Africa (Meyer, **1959**), and from decaying military materiél in a number of tropical areas (Reese et al., **1950**).

Botryodiplodia theobromae has been known to occur on banana since 1913 when Ashby (**1913**) reported finding it associated with a rhizome disease in Jamaica. Wardlaw (**1931**) isolated the fungus from decaying banana fruit in Jamaica, and through inoculation trials demonstrated that it was capable of causing rapid decay of banana fruit in storage. Von Becze (**1932**) isolated *B. theobromae* from Jamaican and Brazilian fruit, but did not regard it as a serious rot pathogen, apparently because of its infrequency among the fungi isolated. Roy and Sharma (**1952**) reported that *B. theobromae* attacked most of the table varieties of banana in Punjab and Bihar, and Chona (**1933**) cited it as the cause of pseudostem rot, main stalk (inflorescence axis) rot, and a black or

finger tip [1] rot of fruit in Punjab. Chona (1933) was able to reproduce these diseases with artificial inoculations. Mallamaire (1936) reported *B. theobromae* as the cause of black rot of banana in French Guinea, and Hirai (1938) found this same fungus associated with rot of bananas shipped from Formosa to Japan. Recently, *B. theobromae* has been isolated from decaying "crowns" of boxed banana fruit shipped from Central America to the United States, and inoculation trials have again demonstrated that, under some conditions, *B. theobromae* is a virulent rot pathogen.

The present paper reports the occurrence of *B. theobromae* in the seeds of *Musa* from several localities, and presents some observations on the occurrence of the fungus on banana fruit. Certain aspects of the growth and morphology of the fungus in culture are also discussed.

LITERATURE REVIEW

Botryodiplodia theobromae was originally described by Patouillard (1892) on the fruit of cacao from Ecuador. According to reviews by Petch (1910) and Voorhees (1942), this fungus has been subsequently described under a number of names. Petch (1910) accepted Patouillard's name for the fungus, listing seven synonyms, and was among the first to recognize its wide host range. Griffon and Maublanc (1909) placed this species in *Lasiodiplodia*, a name that Petch (1910) considered synonymous with *Botryodiplodia*. Taubenhaus (1915), in a study of *Diplodia* and related genera, reported that, on inoculated sweet potato, *Diplodia gossypii* showed "all of the characteristics of the supposed genera *Lasiodiplodia, Chaetodiplodia, Botryodiplodia* and *Diplodiella*." Similar results were obtained when *Lasiodiplodia tubericola* Ell. & Ev. was inoculated on sweet potato. Taubenhaus (1915) concluded from these results that all species placed in these genera are congeneric and should be assigned to *Diplodia*. Nowell (1923) referred *B. theobromae* to *Diplodia*, listing seven synonyms. Shear (1933) and Stevens (1941) believed *B. theobromae* to be identical with *Diplodia natalensis* Pole-Evans, the cause of citrus black rot, under which Stevens (1926) also placed *Diplodia gossypina* Cooke as a synonym. Through single ascospore culture, Stevens (1926) and Stevens and Wilcox (1925) demon-

[1] Note on terminology: In this paper, the individual banana fruit is referred to as a "finger"; and the short stalk by which it is attached as the "finger stalk"; several fingers constitute a "hand." The fingers are attached to a crescentic "cushion" or "crown," which projects from the "main stalk" of the whole banana bunch, or "stem." For boxing, the "crown" and attached "fingers" are removed from the "main stalk" and the fruit is packed as "hands."

strated that *D. natalensis* is an imperfect state of *Physalospora rhodina* (Berk. & Curt.) Cooke. Voorhees (**1942**) examined isolates of *P. rhodina* and its imperfect states collected from many different hosts throughout the world. He listed sixteen synonyms for *P. rhodina*, among them *B. theobromae*. The most recent revision involving this fungus is that of Zambettakis (**1954**) who followed Griffon and Maublanc (**1909**) in placing it in the genus *Lasiodiplodia*. Zambettakis (**1954**) lists thirty-seven synonyms for *L. theobromae*, but does not include *D. natalensis* or *D. gossypina* among them. A summary of the synonymy of this species, as given by various authors, is presented in TABLE I.

Eddins and Voorhees (**1933**) suggested that Diplodiae having their perfect stage in *Physalospora* be referred to by the name of the perfect

TABLE I

LIST OF SYNONYMS OF *Botryodiplodia theobromae* AS GIVEN BY SEVEN AUTHORS

Synonym	Author
Botryodiplodia batatae Henn.	Zambettakis (1954)*
B. carpophila Petr.	Zambettakis*
B. cerebrina Sacc.	Zambettakis*
B. creba (Sacc.) Petr.	Zambettakis*
B. diplocarpa Ell. & Ev.	Zambettakis*
B. elasticae Petch	Petch (1910), Cook (1913), Nowell (1923),** Zambettakis,* Voorhees (1942)***
B. gossypii Ell. & Bart.	Voorhees***
B. manihotis Syd.	Zambettakis*
B. manihoticola Petr.	Zambettakis*
B. phaseolina (Sacc.) Petr. & Syd.	Zambettakis*
B. saccharina Died.	Zambettakis*
B. tubericola (E. & E.) Petr.	Voorhees***
Chaetodiplodia arachidis Maubl.	Zambettakis*
C. coffeae Zimm.	Zambettakis*
C. grisea Petch	Zambettakis,* Voorhees,*** Nowell,** Petch
C. sobraliae Henn.	Zambettakis*
C. vanillae Zimm.	Zambettakis*
Diplodia arachidis Petch	Zambettakis*
D. cacaoicola Henn.	Zambettakis,* Cook, Nowell,** Petch, Voorhees***
D. cinnamoni Da Camara	Zambettakis*
D. cococarpa Sacc.	Zambettakis*
D. cocophila Cke.	Zambettakis*
D. coffeae Henn.	Zambettakis*
D. coffeicola Zimm.	Zambettakis*
D. coffaeiphila Speg.	Zambettakis*
D. creba Sacc.	Zambettakis*

* Given as synonyms of *Lasiodiplodia theobromae*.
** Given as synonyms of *Diplodia theobromae*.
*** Given as synonyms of *Physalospora rhodina*.

TABLE I—(*Continued*)

Synonym	Author
D. gossypina Cke.	Stevens,** Voorhees***
D. hesperidica Speg.	Zambettakis*
D. mangostanae Henn.	Zambettakis*
D. manihoti Sacc.	Zambettakis*
D. musae Died.	Zambettakis*
D. natalensis Evans	Stevens (1941),** Voorhees,*** Shear (1933)**
D. paradisiana (Mont.) Wr.	Zambettakis*
D. phaseolina Sacc.	Zambettakis*
D. radula B. & Br.	Zambettakis*
D. rapax Massee	Nowell,** Cook, Voorhees,*** Zambettakis*
D. solanicola Sacc.	Zambettakis*
D. theobromae (Pat.) Nowell	Voorhees***
D. tubericola Ell. & Ev.	Zambettakis*
D. tubericola (Ell. & Ev.) Taub.	Zambettakis,* Voorhees***
D. zebrina Petch	Zambettakis*
Lasiodiplodia nigra Appel & Laubert	Voorhees,*** Cook, Petch, Nowell,** Zambettakis*
L. theobromae Griff. & Maubl.	Voorhees,*** Cook, Petch, Nowell,** Zambettakis*
L. triflorae Higgins	Voorhees***
L. tubericola Ell. & Ev.	Voorhees***
Macrophoma vestita Prill & Del.	Petch, Nowell,** Cook
Nematodiplodia laelio-cattleyae Sib.	Zambettakis*
Thyridaria tarda Bancroft	Cook

stage. In a later publication, however, Voorhees (**1942**) noted that "there are at least three distinct species of *Physalospora* that are not distinguished by their *Diplodia* stages. Thus, it is entirely possible that many of the *Diplodia* forms included under *P. rhodina* have their sexual stages in undescribed species of *Physalospora* and other genera." While *B. theobromae* on banana is probably an imperfect form of *P. rhodina,* the perfect stage has never been collected on that host. In the absence of conclusive evidence that the banana pathogen is genetically related to *P. rhodina,* it appears preferable to refer to it as *B. theobromae,* the name by which it is best known in banana pathology.

RESULTS AND OBSERVATIONS

Occurrence of B. theobromae in Seeds of Musa.—The occurrence of *B. theobromae* as a seed-borne fungus in *Musa* was detected during attempts to germinate aseptically seeds of *M. balbisiana* and *M. textilis.* Certain lots of seeds were found to be nearly 100% infected. The fungus was not eliminated by immersing the seeds in solutions of

$Ca(OCl)_2$ (10 g $Ca(OCl)_2$ in 150 ml distilled water) or NaOCl (5% commercial Clorox) for periods of 10 to 30 minutes, although in most cases, all other microorganisms were killed by these treatments. In some seed lots the presence of *B. theobromae* was detected only in surface-sterilized seeds, suggesting that in seeds which had not been so treated, growth of the fungus was inhibited or obscured by other microorganisms.

TABLE II

OCCURRENCE OF *Botryodiplodia theobromae* IN SEED OF SEVERAL SPECIES OF *Musa* FROM VARIOUS LOCALITIES

Collection number	Species	Origin	Infected* seed
2-7573	*Musa* sp.	North Borneo	−
2-7568	*Musa* sp.	North Borneo	−
2-7583	*M. angustigemma*	North Boreno	−
2-7584	*M. balbisiana*	North Borneo	+
2-7592	*M. reflexa*	North Borneo	+
2-7594	*M. acuminata* subsp.	North Borneo	−
2-7596	*M. acuminata*	North Borneo	−
2-7597	*M. textilis*	North Borneo	−
2-7600	*M. acuminata* var. *microcarpa*	North Borneo	−
2-7621	*M. borniensis*	Sarawak	−
2-7622	*M. acuminata* var. *microcarpa*	Sarawak	−
2-7631	*M. violascens*	Sarawak	−
2-7651	*M. acuminata* var. *microcarpa*	Sarawak	−
2-7667	*M. violascens*	Malaya	−
2-7675	*M. acuminata* var. *malaccensis*	Malaya	−
2-7678	*M. acuminata* var. *malaccensis*	Malaya	−
2-7681	*M. acuminata*	Malaya	+
2-7685	*M. acuminata*	Malaya	−
2-7686	*M. gracilis*	Malaya	+
2-7687	*M. acuminata* var. *microcarpa*	Malaya	+
2-7688	*M. gracilis*	Malaya	−
	M. textilis	Honduras	+
	M. balbisiana	Honduras	+
	M. balbisiana	Panama	+

* + = infected seed.

Seeds of several species and subspecies of *Musa* were examined for presence of the fungus (TABLE II) by plating seeds surface-sterilized in $Ca(OCl)_2$ or NaOCl solutions on aqueous agar. The fungus was found in seeds of five species or subspecies, obtained from Panama, Honduras,[2] North Borneo [3] and Malaya.[3] The widespread occurrence of the fungus in seeds suggests that *B. theobromae* may be a common cause of decay of seeded banana fruit in nature, inasmuch as the fungus probably invades the seeds from the decaying fruit pulp.

[2] Obtained through the cooperation of Drs. R. H. Stover and B. H. Waite.

[3] Collected and identified by Mr. Paul Allen.

To determine the depth of infection and the parts of the seeds which were invaded by the fungus, a number of surface-sterilized seeds of *Musa balbisiana* from Honduras were dissected aseptically and various seed parts plated separately. In thirty seeds examined by this method, the fungus developed almost exclusively from the seed coat and micropylar plug (FIG. 1). In two seeds, the fungus was present in the chalazal mass, but it was not found in the endosperm or embryo of any of the seeds examined. A large number of embryos of *M. balbisiana* have been grown in embryo culture (Cox et al., 1960), and even though these were obtained from seed lots in which a high percentage of the seeds were infected by *B. theobromae*, the fungus never developed from excised embryos. All the evidence thus indicated that the infection

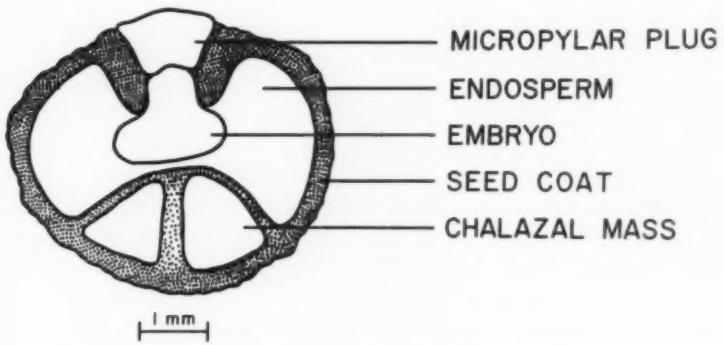


FIG. 1. Diagrammatic longi-section of the seed of *Musa balbisiana*.

was limited to the seed coat or micropylar plug, but that it was sufficiently deep so as to be unaffected by surface sterilants.

Survival of *B. theobromae* in seeds which had been immersed in solutions of strong acids or alkali for various periods of time (TABLE III) further indicated that the infections were deep-seated. Growth of the fungus from seeds immersed in strong acids was slower than from those treated with dilute reagents, suggesting that strong reagents may have destroyed the fungus in the outer portions of the seed coat. Failure of other surface sterilants (i.e., 70% alcohol, 1:1000 benzalkonium chloride, 1% H_2O_2) to eliminate the fungus offered further evidence that infection was deep-seated. Radiation treatments, in which infected seeds were exposed to gamma radiation doses as high as 250 Krad also failed to kill the fungus, in agreement with data reported by Beraha et al. (1960) for *D. natalensis*.

Thermal Death of B. theobromae.—Botryodiplodia theobromae caused rapid decay of seeds in aseptic conditions, although it did not appear to seriously affect germination under greenhouse conditions, and heat treatments were investigated as a possible means of freeing seeds of infection.

In preliminary experiments, thermal death of the fungus on agar slants was determined. The nature of the resistant propagule in seeds was unknown, but inasmuch as pycnidia were never observed on seeds, it seemed probable that the fungus survived as chlamydospores or other resistant hyphal units. To test thermal tolerance of all structures pro-

TABLE III

SURVIVAL OF *Botryodiplodia theobromae* IN CHEMICALLY TREATED SEEDS OF *Musa balbisiana*

Solvent	Dilution	Immersion time (min.)				
		15	30	60	120	240
HCl	conc. (38%)	−	+	+	−	−
	1:1	+	+	+	+	+
	1:5	+	+	−	+	+
	1:10	+	+	+	+	+
NaOH	conc. (30%)	+	+	+	+	+
	1:1	+	+	+	+	+
	1:5	+	−	+	−	+
	1:10	+	+	+	+	+
H_2SO_4	conc. (93–98%)	+	+	+	+	+
	1:1	+	+	+	+	+
	1:5	+	+	+	+	+
	1:10	+	+	+	+	+
HNO_3	conc. (70–71%)	+	+	+	+	−
	1:1	+	+	−	−	−
	1:5	+	+	+	+	+
	1:10	+	+	+	+	+

+ = survival of the fungus.

duced by the fungus, three-week-old cultures, grown on potato dextrose agar slants, and containing mycelium, stroma, pycnidia, and hyaline one-celled spores were exposed to various temperatures for intervals ranging from 7 to 60 minutes. Liquid nutrient medium, preheated to the temperature to be tested, was added to the surface of the slants immediately before they were immersed in a thermostatically controlled water bath. The liquid medium served as a means of checking survival of the fungus and ensured transfer of heat to the culture surface. Under these conditions, the fungus survived exposures of one hour at temperatures up

TABLE IV

THERMAL DEATH OF *Botryodiplodia theobromae* ON AGAR SLANTS

Temperature	Exposure—minutes			
	7	15	30	60
40	+	+	+	+
45	+	+	+	+
50	+	+	+	+
55	+	−	−	−
60	−	−	−	−
65	−	−	−	−
70	−	−	−	−
75	−	−	−	−

+ = growth. − = no growth.

to and including 50° C, but survived only a 7 minute exposure at 55° (TABLE IV). Longer exposures at 55° proved lethal, as did all exposures at higher temperatures.

To determine whether the fungus could be eradicated from seeds with heat treatments without damage to the embryo, seeds of *Musa balbisiana* were immersed in a thermostatically controlled water bath at temperatures ranging from 60 to 80°, for intervals ranging from 10 to 30 minutes. The fungus survived in seeds exposed to 70° for 10 minutes, but longer exposures at 70°, and all exposures at higher temperatures proved lethal (TABLE V). Seeds exposed to the maximum time-temperature combination (80° for 30 min) remained viable. In practice, immersing seeds in water at 75° for 10 minutes has proved effective in killing the fungus without loss of seed viability.

Greater thermal tolerance of the fungus in banana seeds than on agar slants can probably be attributed to poor heat transfer within the

TABLE V

SURVIVAL OF *Botryodiplodia theobromae* IN HEAT-TREATED SEEDS OF *Musa balbisiana*

Temperature	Exposure—minutes				
	10	15	20	25	30
60	+	+	−	+	+
65	+	+	−	+	−
70	+	−	−	−	−
75	−	−	−	−	−
80	−	−	−	−	−

+ = growth. − = no growth.

seed. It is possible, however, that the fungus was present in the seed in a more resistant form than was found on the agar slants.

Occurrence of B. theobromae on Banana Fruit.—The wide distribution of *B. theobromae* in the seeds of *Musa* suggested that the fruit of many species may be attacked by this fungus. Wardlaw (**1931**) noted that mature fruits of "practically all" varieties he observed in Jamaica, including seeded forms, were attacked by this fungus. Meredith (**1961**) reported finding spores of *B. theobromae* in the air over banana plantations in Jamaica, indicating that dispersal is probably by air-borne spores.

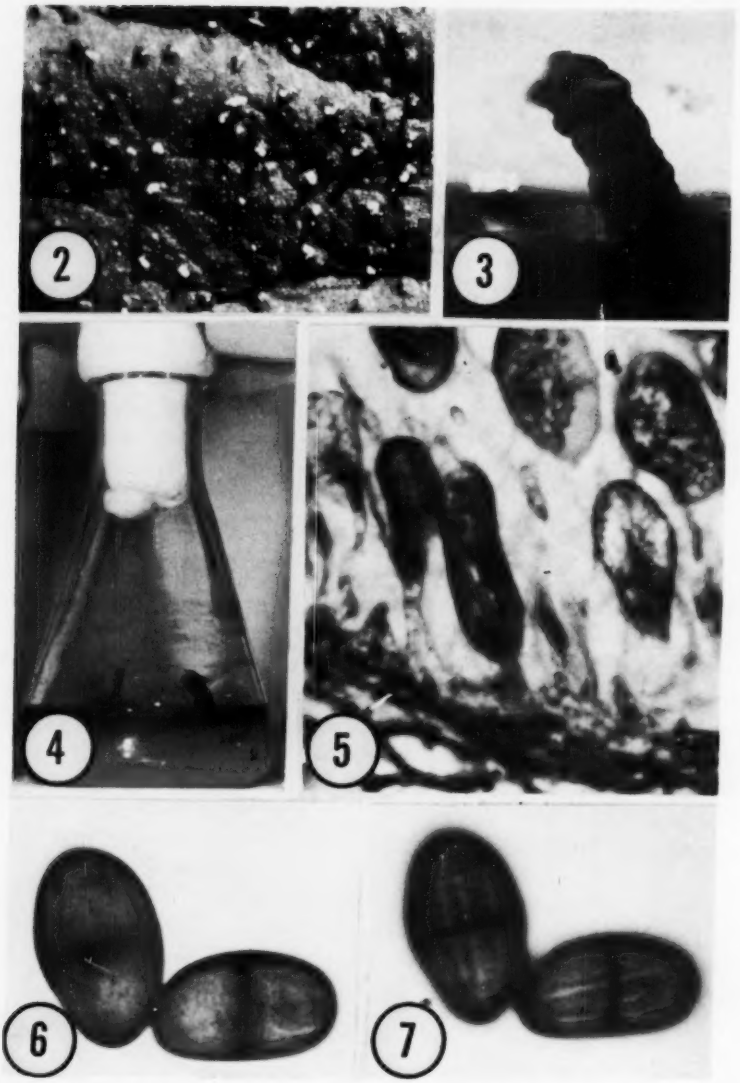
The prevalence of *B. theobromae* on commercial bananas can be readily demonstrated by placing unripe fruit in an environment favorable for rot development. Central American fruit was examined for presence of the fungus by this method during the period of April to August, 1961. Thirty to forty fingers, taken at random from upper, middle, and lower hands, were removed from each of the stems examined and washed for ten minutes in a 5% Clorox solution. The fruit was then placed on moist toweling in culture dishes and incubated at temperatures of 30–32° for a week. Under these conditions, 5 to 30% of the fingers from a given stem developed *Botryodiplodia* rots, often in combination with *Gloeosporium musarum* Cke. & Massee. Rots rarely developed on surface-sterilized fruit from the same stems when maintained at 21°, a temperature slightly higher than that recommended for banana ripening. Use of low temperature during shipping and storage was recommended by Wardlaw and McGuire (**1932**) and Hirai (**1938**), for preventing losses due to *B. theobromae,* and the widespread use of refrigerated holds and storage rooms may explain why *B. theobromae* is not a more serious and destructive pathogen in commercial bananas.

At high temperatures, *Botryodiplodia* rots developed most frequently from the severed finger necks, although in some cases rots developed from the flower end. The latter type of rot suggested that the fruit may have been invaded through the decaying perianth, a supposition supported by the frequent isolation of *B. theobromae* from dead flower tissue in Colombia (W. R. Phelps; unpublished data). Wardlaw (**1931**) reported flower tip rot as common on Jamaican fruit. The source of inoculum on surface-sterilized fruit was not determined, but it is probable that some spores were not killed by the Clorox treatment. The possibility of localized latent infections cannot be excluded, however.

Botryodiplodia theobromae was isolated only occasionally from decaying crown tissues in shipments of boxed banana fruit from Central

America. But while it was not the most prevalent cause of this disease, *B. theobromae* was capable of causing severe and rapid rot on artificially inoculated crowns. When hands of green Lacatan bananas were inoculated by spreading mycelial suspensions of *B. theobromae* on the newly exposed crown surface, severe rot developed within eleven days. In these experiments, fruit was held at temperatures of 14° for six days, treated with ethylene on the seventh day, and held for five additional days at 18°. Under these conditions, the fungus penetrated the crowns, and invaded the pedicels and pulp of the individual fingers. Infection was accompanied by a premature ripening of the fruit, an effect which had been previously noted by Wardlaw and McGuire (**1932**). These authors investigated the possibility that the premature ripening effect might be due to the release of diastase enzymes by the fungus. They found, however, that the portions of the fruit killed and invaded by the fungus still contained starch, while tissues remote from the inoculum had undergone premature ripening. They concluded that, while acceleration of the saccharification process in infected fruit was related to the presence of the fungus, it could not be referred to direct action of enzymes diffusing from it.

Cultural and Morphological Observations on the Fungus.—The morphology of *B. theobromae* has been described by several authors (Howard, **1901**; Taubenhaus, **1915**; Pole-Evans, **1910**; Wardlaw, **1932**; and Zambettakis, **1950**). All isolates examined during the present studies agreed in principal points with descriptions as given by these authors, and were similar in gross cultural appearance, producing a greenish-black floccose colony on glucose-peptone or potato-dextrose media. All isolates produced conidia of similar size (22–33 × 13–15 μ) and with the characteristic longitudinal striations (FIGS. 6, 7); however, they varied considerably in growth characteristics and sporulating ability. Most were slow to sporulate in culture, and an isolate from Honduras soil produced few spores, even in very old cultures. Mature, two-celled, brown spores were found only in cultures two to three weeks old. Some isolates produced only simple pycnidia, while others formed massive, columnar stromata containing several pycnidia. The large, columnar stromata often developed from infected seeds on aqueous agar (FIGS. 3, 4). On infected fruit, however, only the simple pycnidia were found (FIG. 2). Wardlaw (**1932**) found that morphology of the pycnidium was greatly influenced by nutrition, and substrates high in carbohydrates favored development of the large complex stromata. Voorhees (**1942**) considered the morphology of the pycnidium and stroma to be extremely



FIGS. 2–7. *Botryodiplodia theobromae*. 2. Pycnidia on the peel of decayed banana. Note extrusion of hyaline 1-celled spores, × 10. 3, 4. Columnar stromata developing from infected seed of *Musa balbisiana*. (FIG. 3—× 3; FIG. 4—× 3). 5. Longi-section of pycnidium showing immature conidia on short conidiophores, × 1000. 6, 7. Mature 2-celled conidia as seen in optical section (FIG. 6) and in surface view (FIG. 7), × 1000.

variable, but found conidial shape and size to be relatively constant characteristics.

Optimum temperature for growth of *B. theobromae* ranged from 27–30°, and most isolates produced some growth over the range of 15 to 37°. At temperatures of 37°, some isolates produced intense red pigments, a phenomenon previously noted by Stevens and Wilcox (**1925**) and Voorhees (**1942**). The fungus grew rapidly at 27° and covered a 9 cm Petri dish within three days. The spores likewise germinated quickly; five hours after sowing, spores with germ tubes over 100 μ in length were observed.

In an attempt to obtain the ascigerous stage, twenty-seven isolates of *B. theobromae* from various localities were paired with an imperfect state of *P. rhodina*, originally isolated from citrus and obtained from the American Type Culture Collection (#10936). None of the pairings yielded perithecia. Voorhees (**1942**) attempted to obtain the perithecial state of *P. rhodina* by pairing in all combinations the cultures derived from the ascospores of a single ascus, but he was unsuccessful. This suggests that special cultural conditions may be needed.

Voorhees (**1942**) recognized two types of reactions in paired cultures, intermingling and aversion. He interpreted the aversion reaction as evidence for the existence of physiological races. By pairing cultures derived from the spores of a single ascus, he showed that the genes controlling the reaction segregated in a 1:1 ratio at meiosis, indicating a possible sexual phenomenon. Of the pairings made during the present investigation, all but two gave the aversion type reaction with the isolate from citrus. Isolates giving the intermingling reaction with the citrus isolate, presumably indicating a close relationship, were obtained from Panama and North Borneo.

DISCUSSION

Botryodiplodia theobromae is a less serious cause of wastage in banana fruit than *Gloeosporium musarum* Cke. & Massee or *Thielaviopsis paradoxa* (de Seynes) v. Höhn., which are probably the most common and destructive pathogens of banana fruit. It is sufficiently common and destructive, however, to merit the attention of fruit pathologists.

The fungus develops rapidly on infected fruit and, in contrast to those organisms which cause localized lesions, develops evenly around the finger. Infected fruit is totally rotted within a few days after the first signs of infection. Invasion is accompanied by a darkening of the peel, and the development of a characteristic water-soaked appearance.

The pulp becomes discolored, soft and semiliquid. Copious mycelial development may or may not occur, depending on the humidity of the storage chamber (Wardlaw, **1931**). In the final stages of decay, simple pycnidia develop on the peel surface of the infected fruit.

Low temperature storage, recommended by Wardlaw and McGuire (**1931**) and Hirai (**1938**), appears to be adequate for control of *Botryodiplodia* rots in commercial fruit shipments, since the fungus was only rarely observed on fruit maintained at temperatures near those recommended for ripening.

The occurrence of *B. theobromae* as a seed-borne organism in *Musa* is consistent with its role as a fruit rot fungus, inasmuch as it probably invades the seed from the decayed fruit pulp. Its widespread distribution in seeds indicates that it may play an important part in the decay of unharvested bananas in nature.

B. theobromae appeared to die out in infected seeds after prolonged storage. Seed lots which were heavily infected when first examined were found to be free of infection after a year in storage. Death of *B. theobromae* in cultures stored under oil also appears to occur frequently (E. G. Simmons; personal communication), suggesting that the organism may not survive for long periods in a quiescent state. If this observation is borne out by future investigations, storage may prove to be a simple method of eliminating the fungus from infected seeds.

Botryodiplodia theobromae is now known to occur in the seeds of at least four tropical plants (Noble et al., **1958**). Its wide host range and its frequent occurrence on decaying fruit of many other plant species suggest that it may have a wider distribution as a seed-borne fungus.

SUMMARY

Botryodiplodia theobromae Pat., a facultative parasite on a wide range of tropical plants, is often associated with decaying fruit of banana, and frequently invades the seeds of seeded species. The fungus has been isolated from surface-sterilized seeds of five species of *Musa*, obtained from such widely separated points as Panama, Honduras, North Borneo and Malaya. In seeds of *M. balbisiana*, the fungus was found to reside primarily in the seed coat or micropylar plug. The fungus survived in seeds treated with various surface-sterilants; however, heat treatment by immersing seeds in water at 75° for 10 minutes killed the fungus without injury to the seed. On agar slants, thermal death of the fungus occurred after a seven minute exposure to 55°. Prevalence of the fungus in commercial fruit shipments and certain aspects of the

growth and morphology of the fungus in culture are discussed, and it is suggested that *B. theobromae* may be a frequent cause of decay of bananas in nature.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Dr. George L. Greene for permission to use the photograph shown in FIG. 2, and to Dr. W. R. Phelps for permission to use his unpublished data. The inoculation trials described in this paper were carried out in cooperation with Dr. Greene, and will be described in more detail in a paper now in preparation.

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TWO SPECIES OF CONIDIOBOLUS OFTEN FORMING ZYGOSPORES ADJACENT TO ANTHERIDIUM-LIKE DISTENTIONS

CHARLES DRECHSLER [1]

(WITH 10 FIGURES)

Two readily culturable entomophthoraceous fungi that first appeared on Petri plates of maize-meal agar canopied with moist filter paper to which was affixed some partly decayed plant material are herein described as new species of *Conidiobolus*. The 2 fungi are advantageously discussed together, for although they differ enough to be readily distinguished from each other, both show in an antheridium-like distention often adjoining their zygospores a morphological modification prominently set forth in the figures and description of *C. brefeldianus* Couch (**1939**), and therefore alike invite comparison with that species. As the original culture of *C. brefeldianus* has unfortunately been long extinct, comparison must be made mainly with Couch's detailed characterization. Supplementary attention is devoted especially to an isolate from central Florida (FIG. 1, A) that I hold properly referable to *C. brefeldianus* and to a rather similar but perhaps not conspecific isolate from northern Wisconsin (FIG. 1, B). Opportunity has been lacking for comparison of my cultures with the *"Conidiobolus? brefeldianus"* recently mentioned by Korf (**1960**) as having been isolated in Highlands, North Carolina, from apothecia of *Peziza proteana* (Boud.) Seav. f. *proteana*. Type cultures of the 2 new species have been transmitted to the American Type Culture Collection, Washington, D. C., and dried material of each has been given to the National Fungus Collections, Plant Industry Station, Beltsville, Maryland. To compensate partly for the very obvious shortcomings of the dried material, figures of reproductive bodies prepared from mounts of living material are supplied in numbers sufficient, it is hoped, to indicate the more usual variations in size and shape.

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Conidiobolus polyspermus Drechsler, sp. nov. (πολύσπερμος, abounding in seed)

In materiis macris oculo nudo saepe aliquid inconspicuus, sed in materiis pinguioribus multo facilius visibilis. Hyphae assumentes incoloratae, mediocriter ramosae, primo filiformes, prope marginem mycelii crescentis plerumque 6–13 μ latae, ibi ex cellulis 40–300 μ longis constantes, hic illic ramos saepius 2–9 μ latos emittentes, postea medio mycelii ex magna parte in cellulas inflatas disjunctas 35–100 μ longas et 12–25 μ latas gradatim transeuntes; hyphae vagae vulgo 40–250 μ longae, 2–4 μ latae. Hyphae fertiles incoloratae, vulgo simplices, in aerem saepius 30–125 μ ad lucem protendentes, plerumque 7–18 μ latae; conidia violenter absilientia, incolorata, globosa, in toto plerumque 15–55 μ longa, 12–48 μ lata, basi papilla saepius 3–15 μ longa et 5–16 μ lata praedita. Zygosporae nunc e copulatione cellularum ejusdem hyphae contiguarum ortae, nunc e copulatione disjunctarum cellularum ejusdem hyphae vel cellularum duarum hypharum oriundae, in maturitate flavidae vel interdum paene incoloratae, leves, globosae vel elongato-ellipsoideae, plerumque 15–48 μ longae, 14–45 μ latae, guttula oleacea 8–30 μ crassa praeditae, muro 1.2–5.5 μ crasso circumdatae.

Habitat in foliis quercorum putrescentibus prope College Park, Maryland. TYPUS: National Fungus Collections No. 71712; American Type Culture Collection No. 14444.

When growing on substratum of low nutrient content usually rather inconspicuous to the naked eye, but on richer substratum becoming more readily visible, often without production of much aerial mycelium. Assimilative hyphae colorless, at first filamentous, at forefront of a growing mycelium maintaining a rather uniform width mostly between 6 and 13 μ, here divided into cells 40 to 300 μ long, and putting forth branches mostly 2 to 9 μ wide, later in positions behind the forefront usually in large part undergoing conversion into swollen segments 35 to 100 μ long and 12 to 25 μ wide; migratory hyphae often appearing tardily, mostly 40 to 250 μ long and 2 to 4 μ wide. Conidiophores colorless, commonly unbranched, extending 30 to 125 μ into the air toward the main source of light, mostly 7 to 18 μ in greatest width, bearing usually a single conidium; conidia springing off forcibly, colorless, mostly 12 to 48 μ in greatest width, 15 to 55 μ in total length inclusive of an abruptly protruding basal papilla usually 3 to 15 μ long and 5 to 16 μ wide at its attachment. Conjugation sometimes occurring directly between 2 contiguous segments of the same hypha and sometimes either between neighboring segments of the same hypha or between segments of 2 neighboring hyphae after production of a connecting branch; zygospore formed wholly within one gametangium and in immediate proximity usually to a distention of the other gametangium, at maturity often noticeably yellowish but sometimes nearly colorless, always smooth, generally globose or somewhat elongated-ellipsoidal, mostly 15 to 48 μ long and 14 to 45 μ wide, containing a reserve globule 8 to 30 μ in diameter, surrounded by a wall mostly 1.2 to 5.5 μ thick.

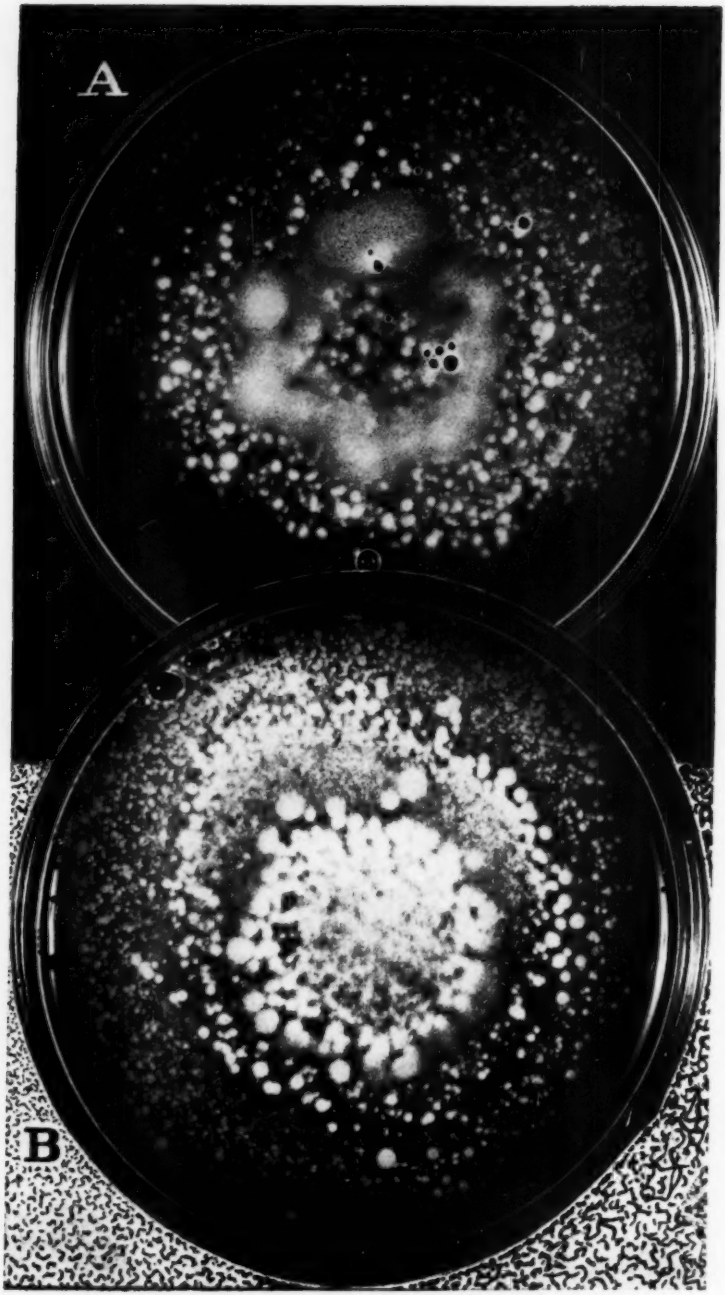

FIG. 1.

Conidiobolus polyspermus was discovered in several Petri plates of maize-meal agar that had been canopied on July 26, 1955, with moist filter paper holding attached some fine detritus sifted from leaf mold newly collected in a wooded area near College Park, Maryland, occupied mainly by white oak (*Quercus alba* L.) and red oak (*Q. rubra* L.). The Petri plates which quite unexpectedly permitted development of many mycelia of *Basidiobolus meristosporus* Drechsler (**1956a**, p. 657) had been incubated at a temperature of 27° C. Not surprisingly they had permitted development also of the ubiquitous *Delacroixia coronata* (Cost.) Syd. & Sacc. emend. Gallaud (**1905**), which on the maize-meal-agar substratum differed little in general appearance from *C. polyspermus*, though after several days the 2 fungi were readily distinguished under the microscope owing to their production of very dissimilar resting spores.

In young maize-meal-agar plate cultures of *Conidiobolus polyspermus* growth of a mycelium is achieved at the advancing margin through apical elongation of hyphae commonly 6 to 9 μ wide (FIG. 3, A). On potato-dextrose agar the elongating hyphae (FIG. 3, B, *a*) at the periphery of a mycelium are noticeably stouter, often measuring 10 to 13 μ in diameter. The long terminal segments in a freshly prepared microscope mount are filled, much as in many congeneric species, with protoplasm of relatively homogeneous texture (FIG. 3, A); and as in congeneric species, too, these segments under microscopical examination soon become divided by cross-walls into shorter cells with vacuolated contents (FIG. 3, B, *a*). Pronounced differences in size of hyphal cells occur in normal development, as is evident when the rather small unicellular migratory hyphae (FIG. 3, B, *b*) in aging tube cultures are compared with the massive resting cells (FIG. 3, C–H) often formed abundantly in Petri plate cultures.

Conidiophores (FIG. 3, I–N) of *Conidiobolus polyspermus* are extended freely from hyphal segments that may be either procumbent on the surface of the substratum (FIG. 3, I, *s*; J, *s*; M, *s*; N, *s*) or submerged under the surface (FIG. 3, K, *s*; L, *s*). Unlike the shaft-like

FIG. 1. Two *Conidiobolus* isolates grown for 72 hours on Petri plates of potato-dextrose agar confined under an inverted battery jar at 25° C; × 1. A, Isolate considered to be identical with *C. brefeldianus*; it was obtained from detritus of decaying grass leaves taken from a roadside near Saint Petersburg, Florida, on April 15, 1959. B, Isolate that agrees somewhat less closely to *C. brefeldianus* and may belong to a separate species; it was obtained from decayed remnants of a forget-me-not (*Myosotis* sp.) stem taken from marshy ground near Park Falls, Wisconsin, on November 18, 1954.

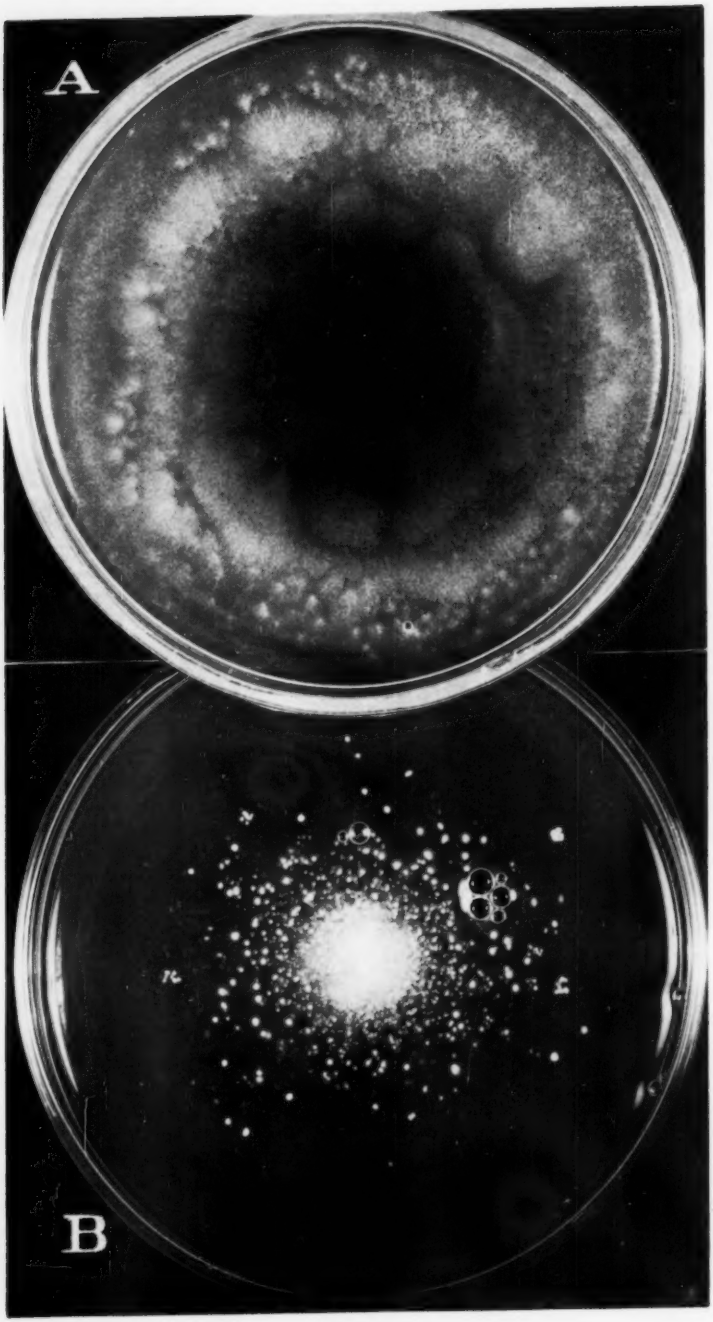

FIG. 2.

cylindrical conidiophores of *Delacroixia coronata*, *C. firmipilleus* Drechsler (**1953b**), and *C. chlamydosporus* Drechsler (**1955**) they are often noticeably broadened some little distance—usually about 10 μ—below the attachment of the conidium (FIG. 3, I–L), but in scattered instances they may be widest at the place of attachment (FIG. 3, M) or as much as 20 to 25 μ below the protruding distal septum (FIG. 3, N). Usually when this septum is completed (FIG. 3, M) by deposition of a portion of membrane at its apex, all except a rather small remnant of protoplasm will have migrated into the conidium. Some denuded conidiophores, however, retain considerable protoplasm (FIG. 3, N) and accordingly are capable either of growing out vegetatively or of resuming asexual reproductive development.

Soon after the conidium of *Conidiobolus polyspermus* has been fully delimited, it springs off forcibly through sudden eversion of its basal membrane. The dome-shaped papilla thereby thrust out appears in the detached conidia (FIG. 3, O, *a–o*; FIG. 4, A, *a–v*) rather sharply demarcated from the globose peripheral wall. With respect to outward shape the detached conidia greatly resemble those of *C. brefeldianus*, which, however, would seem from Couch's description to be of generally smaller dimensions. While the conidia of *C. polyspermus* resemble those of *Delacroixia coronata* both in shape and in size, they have never been found transformed into hirsute resting spores. As might be expected from their strong self-propulsion, the conidia of *C. polyspermus* and *D. coronata* are provided with papillae of noticeably greater size than the similarly large conidia of *C. adiacretus* Drechsler (**1953a**), *C. chlamydosporus*, and *C. megalotocus* Drechsler (**1956b**). Many of the very robust conidia produced by *C. polyspermus* in potato-dextrose-agar cultures bear a papilla of broadly rounded hemispherical shape (FIG. 3, O, *i*, *l*, *m*; FIG. 4, A, *a*, *c*, *d*) rather than of the somewhat tapered paraboloid shape usual in the papillae of smaller spores. In numerous instances the hemispherical papilla contains no vacuole and concomitantly the globose interior is often filled throughout with densely granular protoplasm.

Detached conidia of *Conidiobolus polyspermus* often germinate vegetatively by extending into the substratum one (FIG. 4, B) or two (FIG. 4, C) germ hyphae capable of growing out into new mycelia. Often, too, they extend into the air a single hypha (FIG. 4, D–H) that swells at the tip (FIG. 4, I) and forms there a secondary conidium (FIG. 4, J–L)

FIG. 2. Two new species of *Conidiobolus* grown for 72 hours on Petri plates of potato-dextrose agar confined under an inverted battery jar at 25° C; × 1. A, Type culture of *C. polyspermus*. B, Type culture of *C. gonimodes*.

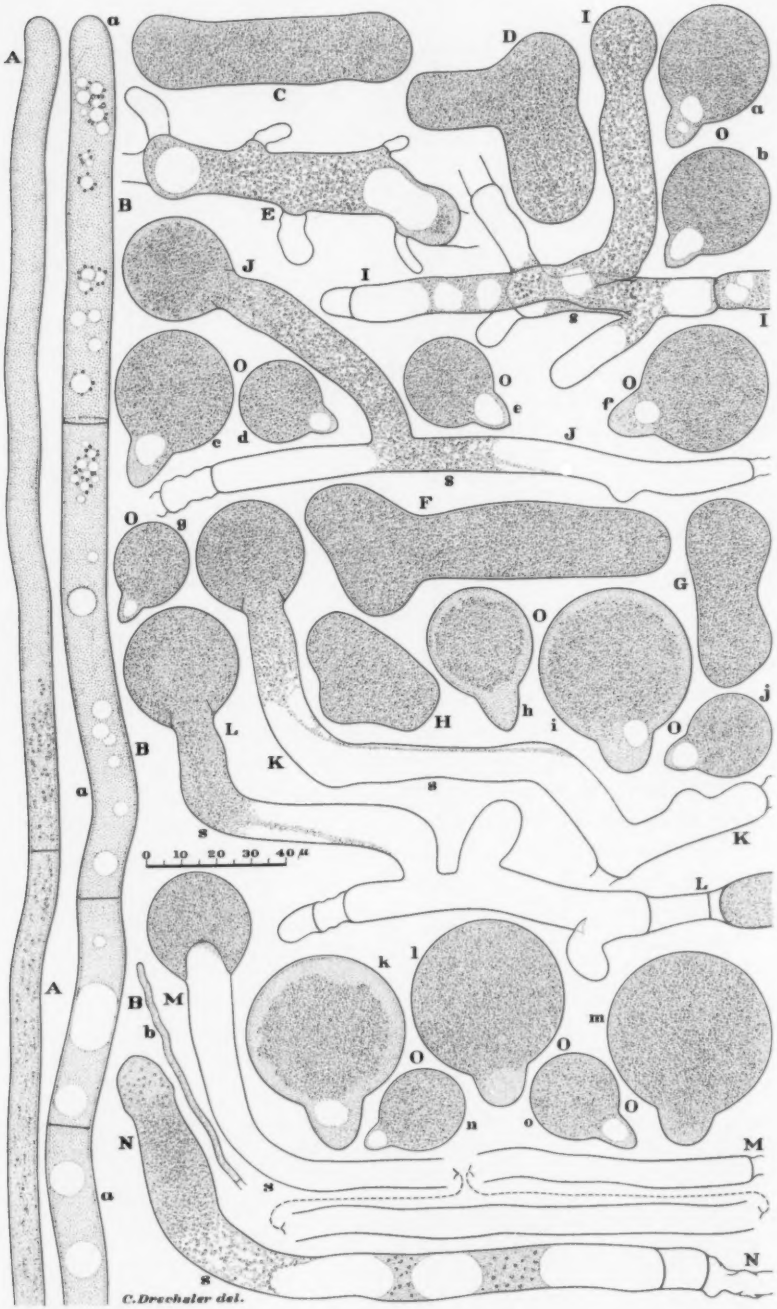
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FIG. 3.

which, like its parent, springs off through sudden eversion of its basal membrane. Continued recurrence of such repetitional development entails progressive reduction in size of the numerous conidia affected. In cultures where evanescence of empty membranes is not too rapid some evacuated conidial envelopes, together with attached tubular remains of germ conidiophores (FIG. 4, M), are found intermixed with thimble-like caps (FIG. 4, N) left by conidiophores of mycelia origin. Multiplicative reproduction by the development of microconidia plurally on short sterigmata has not so far been observed in *C. polyspermus*, though under similar cultural conditions some production of microconidia occurred in the Florida isolate held referable to *C. brefeldianus*.

When *Conidiobolus polyspermus* is grown on maize-meal agar or on lima-bean agar, it forms zygospores in moderate quantity. In potato-dextrose-agar cultures, however, the fungus produces zygospores in extraordinary abundance—a feature which the specific epithet is intended to signalize. In such cultures sexual reproduction is initiated very promptly. Thus, although the Petri plate culture shown in FIG. 2, A, was inoculated only 72 hours earlier, the fungus had become extensively visible in the originally clear potato-dextrose agar, owing in much greater measure to copious development of sexual reproductive apparatus on and under the surface of the substratum than to the moderate formation of conidial apparatus above the surface. In notable contrast, no sexual reproduction whatever had taken place in parallel cultures of the 3 congeneric fungi obtained separately from Florida, Wisconsin, and Illinois (FIG. 1, A, B; FIG. 2, B).

Conjugation often occurs in *Conidiobolus polyspermus* between 2 contiguous hyphal segments (FIG. 5, A; FIG. 6, A) which, through evacuation of adjoining portions of filament, no longer are in direct

FIG. 3. *Conidiobolus polyspermus* drawn with the aid of a camera lucida; × 500. A, Distal portion of an elongating hypha at advancing margin of a mycelium growing in a maize-meal-agar plate culture, drawn within 5 minutes after material was placed on a microscope slide under a cover glass. B, Two filamentous hyphae of unequal width: *a*, distal portion of an elongating hypha at margin of a mycelium in a potato-dextrose-agar plate culture, drawn 50 minutes after material was placed on a microscope slide under a cover glass and exposed to strong illumination from a microscope lamp; *b*, migratory hypha in a maize-meal-agar tube culture 13 days old. C–H, Large distended segments in central area of a mycelium in a potato-agar-plate culture 6 days old. I, Portion of mycelium with a conidiophore bearing a young conidium. J–L, Portions of hyphae, each with a conidiophore showing early stage in formation of wall delimiting a conidium. M, Hypha with conidiophore bearing a fully delimited conidium. N, Conidiophore denuded of its conidium but retaining some protoplasm in living state. O, Detached conidia, *a–o*, showing variations in size and shape. (*s*, surface of substratum.)

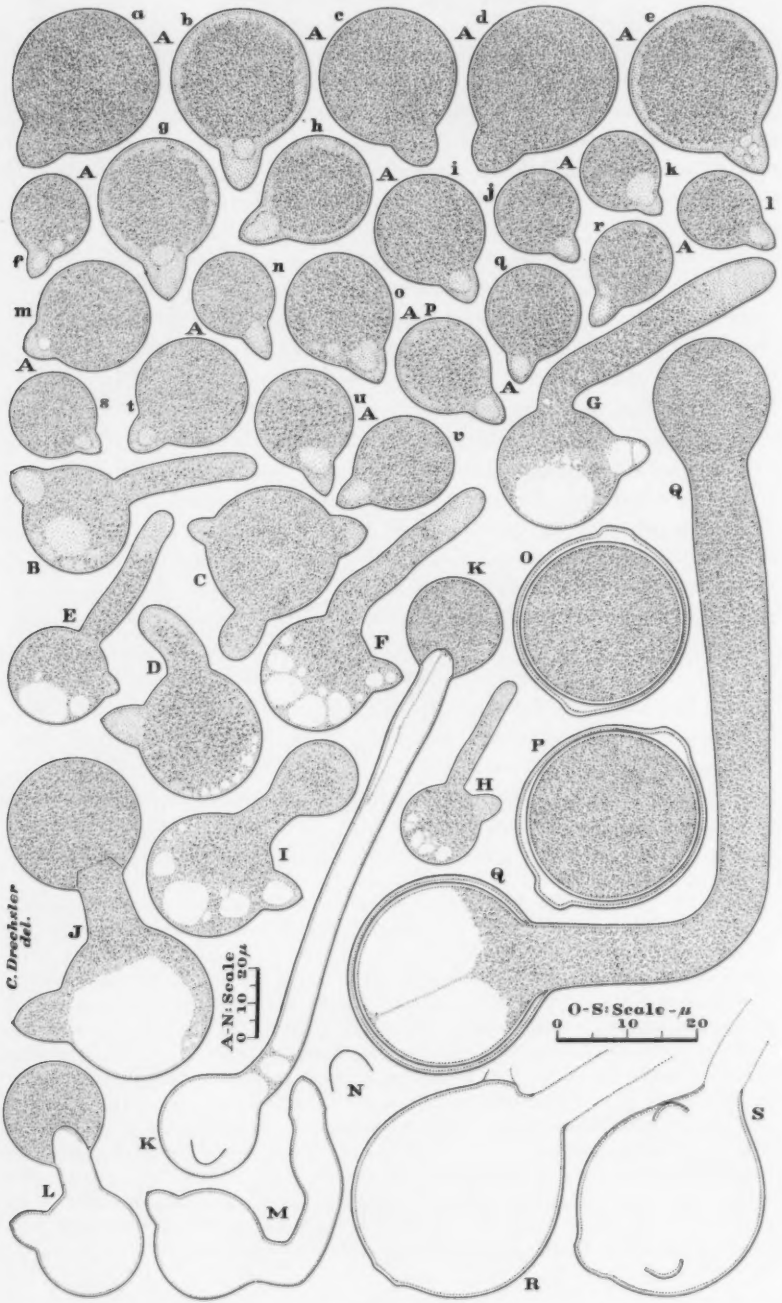

FIG. 4.

contact with any other of their neighbors. As an initial sign of prospective union both segments, especially if they are relatively narrow, may swell rather markedly at their abutting ends. While this preliminary enlargement is taking place, the separating cross-wall seems to remain unchanged (FIG. 6, A). Soon all enlargement ceases in one of the ends, but the other end continues steadily to gain in volume. An opening that permits passage of granular contents now becomes discernible in the flat partition between the increasingly unequal distentions (FIG. 5, A, B), and in one instance 2 such openings could be seen (FIG. 5, C). Beginning, therefore, at a relatively early stage the larger distention receives materials not only from the unmodified portion of the segment to which it belongs but also from the conjoined segment. The migration of protoplasm results in progressive evacuation first of the more distant portions of both segments and later of their closer portions. Successive stages of evacuation are sometimes marked by deposition of retaining walls one after another (FIG. 5, C, D). When the larger distention has received all the contents of both segments, it is walled off proximally and distally as a young zygospore (FIG. 5, E). In somewhat rare instances the contributing segment may retain a substantial remnant of living protoplasm within a cell adjacent to the zygospore (FIG. 5, F). For a while the partition delimiting the zygospore from the contributing segment often appears of unequal thickness (FIG. 5, E, F), its thinner portion corresponding in position to the aperture earlier present between the paired gametangia.

Conjugation often takes place in *Conidiobolus polyspermus* also between 2 hyphal segments that owing to withdrawal of protoplasm from an intermediate portion of filament are no longer contiguous. One of the segments puts forth a branch that makes apical contact with the other segment and unites with it (FIG. 5, G; FIG. 6, B, C). Thereupon the same sequence of developmental events ensues as in pairs of directly

FIG. 4. *Conidiobolus polyspermus* drawn with the aid of a camera lucida; A–N, × 500; O–S, × 1000. A, Detached conidia, *a–v*, showing usual variations in size and shape. B, C, Conidia germinating by production of 1 and 2 germ tubes, respectively. D-H, Detached conidia, each apparently extending a germ conidiophore. I–L, Conidia at different stages in the production of a daughter conidium. M, Empty membranous envelope of conidium and denuded germ conidiophore. N, Thimble-like distal wall of a conidiophore remaining visible after evanescence of the tubular membrane. O, P, After-ripened zygospores in a maize-meal-agar plate culture 59 days old. Q, After-ripened zygospore germinating by production of a conidiophore and conidium in a maize-meal-agar plate culture 54 days old. R, S, Empty membranous envelopes left behind after germination of 2 zygospores in a 60-day-old maize-meal-agar plate culture was completed.

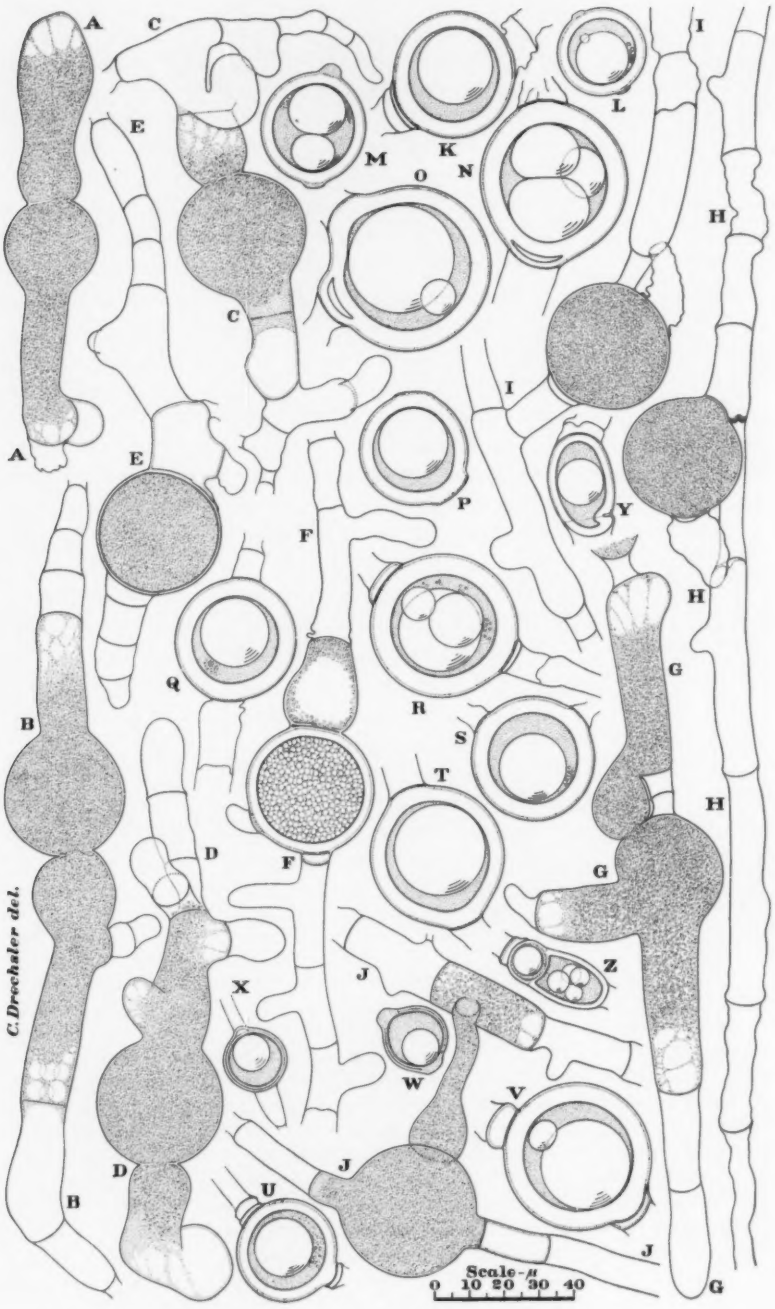
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FIG. 5.

contiguous gametangia, and with the same result that wholly within one gametangium and in immediate proximity to the other a globose distention containing the protoplasm of the entire reproductive unit soon becomes walled off as a young zygospore (FIG. 5, H, I; FIG. 6, D). In scattered instances where the young zygospore is not in axial alignment with the 2 parent hyphal segments but forms a part of the lateral arch (FIG. 5, H) connecting them, it probably was produced distally in the branch. More commonly, however, the zygospore originates in the segment to which the tip of the branch is applied. As a short branch usually applies its tip to the nearest end of the receptive segment, the zygospores produced by paired segments only slightly separated (FIG. 5, G, I); FIG. 6, B) are formed usually in about the same relative position as those produced by paired contiguous segments. A longer branch, on the other hand, may follow a rather circuitous course and apply its tip near the middle of the receptive segment, so that zygospores produced by widely separated consecutive segments often are formed in median positions (FIG. 6, D).

Positional relations are likewise of moment in instances of conjugation between segments belonging to separate hyphae of *Conidiobolus polyspermus*. Segments in 2 neighboring hyphae near the mycelial forefront initiate sexual reproduction by swelling conjointly to form a pair of distentions (FIG. 6, E) side by side, which soon become united laterally. The distention in one segment reaches definitive size about at the time when an aperture becomes discernible in the apposed cohering portions of membrane (FIG. 6, F) and permits migration of protoplasm into the other distention. The latter continues to grow until it has

FIG. 5. *Conidiobolus polyspermus* drawn with the aid of a camera lucida; × 500. A–D, Sexual reproductive units in which conjugation is taking place between 2 adjoining hyphal segments; in C two openings are visible in the wall separating the paired cells. E, Sexual reproductive unit in which a young zygospore has resulted from conjugation of 2 adjoining segments. F, Reproductive unit similarly derived from 2 adjoining hyphal segments; the zygospore here is half mature, and the adjacent antheridium-like cell representing the delimited distention of the contributing segment retains some living protoplasm. G, Young sexual reproductive unit in which conjugation is taking place by means of a short stout branch connecting 2 segments that are consecutive but not contiguous. H, I, Reproductive units in each of which a young zygospore has resulted from conjugation between noncontiguous consecutive segments; in H the zygospore forms a part of the bridging connection and therefore may have originated distally in the branch extended by one segment to make contact with the other. J, Reproductive unit in which conjugation is effected by means of a branch that connects segments belonging to separate hyphae. K–Z, Mature or nearly mature zygospores, showing variations in size, shape, and arrangement of contents.

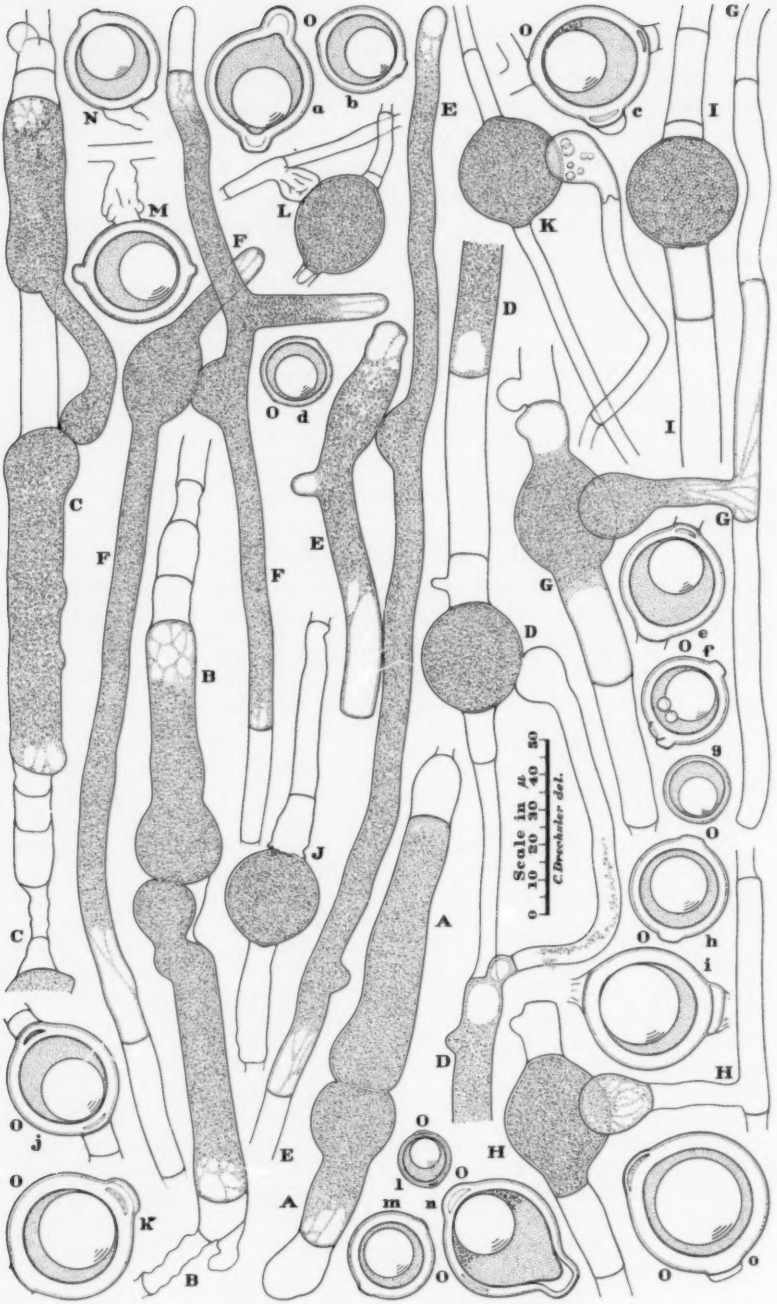

FIG 6

received the protoplasmic contents of the whole reproductive unit and is walled off as a globose young zygospore. Conjugation between paired segments in somewhat more widely separated hyphae is accomplished by means of a connecting branch (FIG. 5, J; FIG. 6, G, H), which commonly is extended from the contributing to the receptive segment. Much as in similar conjugation between separated consecutive segments the zygospore here may develop indiscriminately in a middle, a proximal, or a distal position within the receptive cell.

In sexual reproductive units derived from contiguous hyphal segments the globose zygospore together with an adjacent delimited part of the contributing segment (FIG. 5, F; FIG. 6, I, J) gives somewhat the appearance of an oogonium and adjoining antheridium of the familiar *Pythium ultimum* Trow. Owing likewise to parallelism in arrangement of parts, reproductive units of *C. polyspermus* derived from separate consecutive segments (FIG. 5, I; FIG. 6, D) have a suggestive resemblance to monoclinous sexual apparatus of *P. debaryanum* Hesse. Similarly, reproductive units derived from segments of widely separated hyphae may correspond in appearance to diclinous sexual apparatus of *P. debaryanum*, especially in instances where the distal distention of the connecting branch is set off by a cross-wall (FIG. 6, H, K). Since such a cross-wall is usually formed at a late stage in the migration of protoplasm from the contributing segment into the young zygospore, it would seem of no greater significance than other cross-walls that may be laid down at variable intervals as the filamentous portions of the 2 segments are progressively emptied of their contents.

On reaching its definitive size the young zygospore of *Conidiobolus polyspermus* (FIG. 5, E, H, I; FIG. 6, D, I–L) is surrounded by a thin wall and is filled throughout with densely granular protoplasm. Soon

FIG. 6. *Conidiobolus polyspermus* drawn at a uniform magnification with the aid of a camera lucida; × 500. A, Young sexual reproductive unit composed of 2 adjoining hyphal cells. B, C, Sexual reproductive units in each of which a pair of noncontiguous consecutive segments have become connected by a lateral branch. D, Sexual reproductive unit in which a young zygospore has been formed through conjugation between a hyphal segment and a lateral branch. E, F, Young reproductive units in which long segments of separate hyphae have become united laterally. G, Reproductive unit in which 2 segments belonging to separate hyphae are connected by a lateral branch. H, Same unit as G but drawn 25 minutes later when the terminal distention of the lateral branch had been delimited as an antheridium-like cell. I, J, Reproductive units that have each formed a young zygospore through conjugation between adjoining hyphal segments. K, L, Young zygospores to each of which is attached laterally an empty antheridium-like cell. M, N, Mature zygospores with empty membranous parts attached laterally. O, Mature zygospores, *a–o*, showing variations in size and shape.

thereafter its wall increases in thickness and its contents acquire a globuliferous texture (FIG. 5, F). The ripe zygospores (FIG. 5, K–Z; FIG. 6, M; N; O, *a–o*), like those of various congeneric forms, have a relatively thick wall within which usually a single eccentric reserve globule is very largely or wholly surrounded by protoplasm of nearly homogeneous character. Despite their strongly indurated appearance they are capable of rather early germination under conditions that can hardly be considered exacting. Thus, in a set of maize-meal-agar plate cultures, which, after being inoculated were protected against evaporation and for some time with moderate success also against intrusion of alien microorganisms, zygospores began to germinate spontaneously after about 30 days. Without any water added to the aging cultures germination continued for 25 to 30 days, then being halted because of excessive contamination with species of *Streptomyces*. In zygospores ready for germination (FIG. 4, O, P) a thin outer membrane corresponding apparently to the outer layer of the thick wall present during the period of dormancy was found surrounding rather loosely a thin-walled globose cell filled throughout with densely granular contents. A protrusion of the globose cell pushed its way through the outer envelope and elongated externally as a germ conidiophore (FIG. 4, Q) on which was produced a single self-propelling conidium. Some of the empty parts vanished early from sight; so that when the cultures were 55 days old, more than half of the zygospores originally formed in them were represented by only the thin outer membrane together in some instances (FIG. 4, R, S) with a faintly visible proximal portion of the germ tube.

Though capable of early germination the zygospores of *Conidiobolus polyspermus* are capable also of prolonged dormancy. In maize-meal-agar tube cultures that had been stored 4½ years at temperatures near 8° C more than four-fifths of the zygospores appeared to be in a normal living state after sterile water was added to the air-dry substratum. When portions of the softened substratum were transferred to tubes of sterile agar, new cultures of the fungus were obtained promptly.

Conidiobolus gonimodes Drechsler, sp. nov. (γονιμώδης, fruitful.)

In materiis macris oculo nudo parve visibilis, deinde ex parte albidulus et farinulentus, sed in materiis pinguibus vulgo facile visibilis, albidus, aliquid lanatus. Hyphis assumentibus incoloratis, mediocriter ramosis, primo filiformibus, prope marginem crescentis mycelii plerumque 5–8 μ latis sed interdum usque 13 μ latescentibus, ibi ex cellulis vulgo 40–200 μ longis constantibus, hic illic ramos saepius 2–6 μ rarius usque 10 μ latos emittentibus, deinde ex parte in cellulas disjunctas plerumque 15–200 μ longas 6–25 μ latas tarde transeuntibus; hyphis vagis saepe 50–200 μ longis, 2–4 μ latis. Hyphis fertilibus incoloratis, vulgo simplicibus, rarius parce ramosis,

in aerem saepius 20–80 μ ad lucem protendentibus, 7–12 μ latis, interdum 5–15 μ subter apicem aliquid inflatis; conidiis violenter absilientibus, incoloratis, globosis, in toto 12–39 μ longis, 11–33 μ latis, basi papilla 2–8 μ longa et 2–9 μ lata praeditis, saepe alium conidium saepe aliquot (2–15) microconidia gignentibus; microconidiis incoloratis, globosis vel elongato-ellipsoideis, rectis vel gibberis, primo 8–16 μ longis, 5.5–11 μ latis, identidem alium microconidium in apice hyphae ascendentis 2–15 μ longae 1–2 μ latae ferentibus, ad postremum quandoque tantum 5 μ longis et 5 μ latis, apice interdum pileo glutinoso praeditis. Zygosporis raro et copulatione cellularum disjunctarum ejusdem hyphae ortis, vulgo e copulatione cellularum contiguarum oriundis, saepius flavidulis, levibus, globosis vel elongato-ellipsoideis, plerumque 15–30 μ longis, 11–25 μ latis, guttula oleacea 6–15 μ crassa praeditis, muro vulgo 1–3 μ crasso circumdatis.

Habitat in foliis herbusculae putrescentibus in Chicago, Illinois. TYPUS: National Fungus Collections No. 71711; American Type Culture Collection No. 14445.

On substrata of low nutrient content at first rather inconspicuous to the naked eye but later often becoming more readily visible by forming on the surface a whitish powdery spore deposit; on richer substrata usually conspicuous, whitish, somewhat cottony, often showing many subsidiary mycelia at the margin. Assimilative hyphae colorless, moderately branched, at first filamentous, near the margin of a growing mycelium usually 5 to 8 μ (rarely up to 13 μ) wide, there consisting of segments mostly 40 to 200 μ long, haphazardly giving off branches mostly 2 to 6 μ (rarely up to 10 μ) wide, later in part slowly undergoing conversion into noncontiguous segments mostly 15 to 200 μ long and 6 to 25 μ wide; migratory hyphae commonly 50 to 200 μ long and 2 to 4 μ wide. Conidiophores colorless, usually unbranched but on a rich substratum sometimes sparingly branched, extending 20 to 80 μ into the air toward the main source of light, mostly 7 to 12 μ in greatest width, sometimes slightly inflated 5 to 15 μ below the tip; conidia springing off forcibly, colorless, globose, mostly 11 to 33 μ wide, 12 to 39 μ in total length inclusive of a basal papilla 2 to 8 μ long and 2 to 9 μ wide at its attachment, very often producing a single daughter conidium on a phototropic germ conidiophore or giving rise sometimes in sessile relationship but more commonly on nonphototropic stalks 2 to 12 μ long and 1.5 to 2.5 μ wide to a progeny of 2 to 15 microconidia; microconidia colorless, globose or elongate-ellipsoidal, straight or gibbous, sometimes provided at the tip with a cap of adhesive substance, in the first generation mostly 8 to 16 μ long and 5.5 to 11 μ wide, often giving rise on a stalk mostly 2 to 15 μ long and 1 to 2 μ wide to another microconidium, through continued recurrence of such development sometimes diminishing in length and in width to 5 μ. Zygospores commonly arising from union of contiguous segments of the same hypha, often slightly yellowish, smooth, commonly globose, less often elongate-ellipsoidal, mostly 15 to 30 μ long and 11 to 25 μ wide, containing a reserve globule 6 to 15 μ in diameter, surrounded by a wall 1 to 3 μ thick.

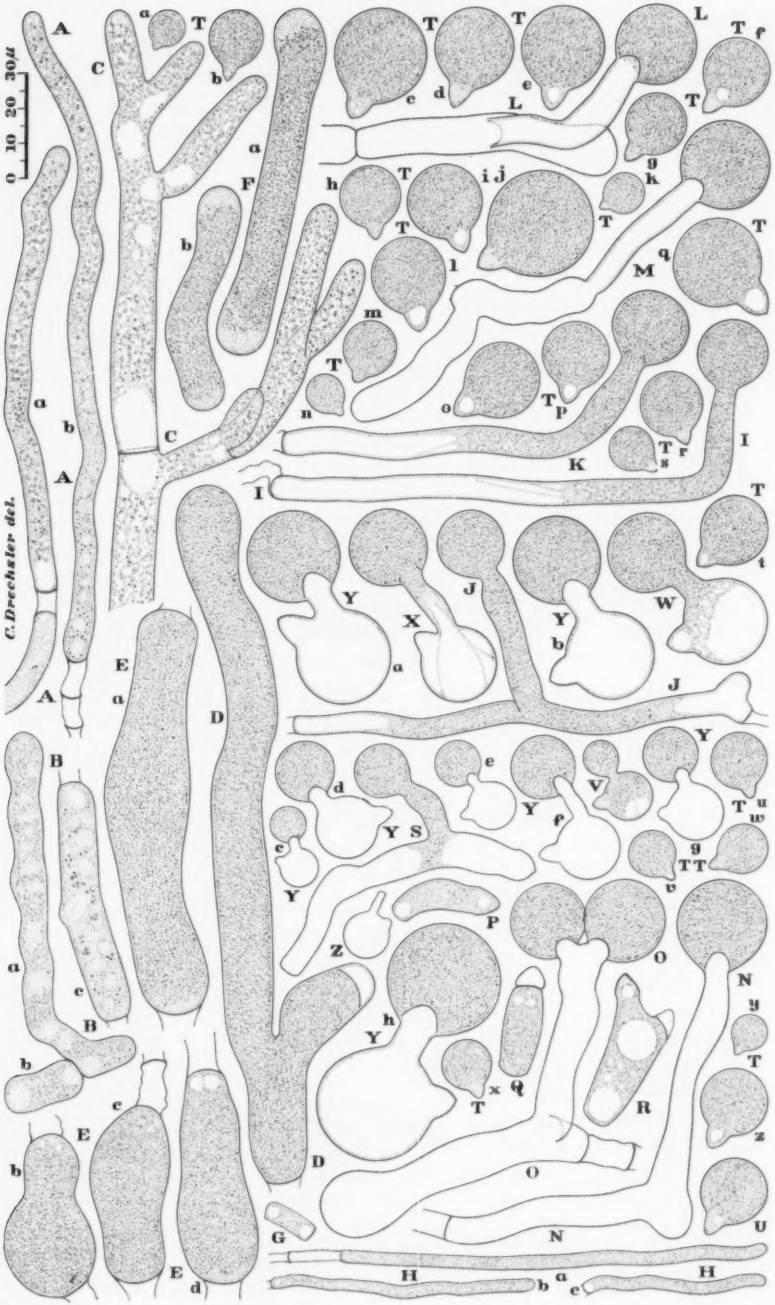

FIG. 7.

Conidiobolus gonimodes was discovered in some Petri plates of maize-meal agar that had been canopied with moist filter paper to which was affixed some fine detritus sifted from decaying herbaceous leaves gathered in an untenanted weedy area in Chicago on November 15, 1954. The submerged mycelium that it pushes through maize-meal agar can be detected with the naked eye when the surface of the culture is viewed at an appropriate angle under a moderately bright light. In Petri plates of potato-dextrose agar, where its growth is readily visible (FIG. 2, B) owing to development of a white downy covering, it shows after 3 days a central area of mycelium surrounded by numerous small mycelia resulting from the germination of individual conidia. The scattered mycelia here seem to merge less freely than in *C. polyspermus*, and in some instances the advance of their peripheral hyphae seems greatly retarded. At an actively advancing mycelial forefront the elongating hyphae (FIG. 7, A, *a*, *b*) are mostly 5 to 8 μ wide, whereas at a retarded forefront the peripheral hyphae (FIG. 7, C) may measure 8 to 13 μ in width and may show unusually close branching. Backward from the border of an expanding mycelium the hyphae (FIG. 7, B, *a–c*) are found composed of segments mostly 7 to 12 μ wide. Segments 13 to 25 μ wide that can be found here and there in aging cultures (FIG. 7, D; E, *a–d*; F, *a*, *b*) are perhaps to be considered rather exceptional in view of the moderate dimensions generally characteristic of the fungus. However, in aging cultures are also found some relatively small hyphal segments (FIG. 7, G)

FIG. 7. *Conidiobolus gonimodes* drawn with the aid of a camera lucida; × 500. A, Terminal segments, *a* and *b*, of elongating hyphae at forefront of an actively growing mycelium. B, Hyphal segments, *a–c*, about 3 mm from margin of a growing mycelium. C, Distally branched stout hypha at forefront of a mycelium where advance was halted. D, Large terminal segment at mycelial forefront where advance was halted. E, Wide intercalary segments, *a–d*, from a culture 34 days old. F, Wide intercalary segments, *a* and *b*, from a culture 20 days old. G, Small intercalary segment from a culture 7 days old. H, Migratory hyphae, *a–c*, in a maize-meal-agar tube culture 14 days old. I, Conidiophore arising from one end of a procumbent hyphal segment. J, Conidiophore arising from a position near middle of a prostrate hyphal segment. K, Conidiophore showing early stage in formation of wall delimiting the conidium. L–N, Conidiophores with conidium completely delimited by dome-shaped wall. O, Distally branched conidiophore bearing 2 fully delimited conidia in a lima-bean-agar culture. P–R, Detached cells formed from distal portions of conidiophores in which some living protoplasm was retained. S, Detached cell of similar derivation that is producing a new conidiophore. T (*a–z*), U, Detached conidia showing usual variations in shape and size. V, W, Detached conidia that are each giving rise to a new conidium. X, Late stage in production of a secondary conidium. Y, Empty conidia, *a–h*, each with an empty germ conidiophore bearing a fully delimited new conidium. Z, Small empty conidial envelope left after daughter conidium has sprung off.

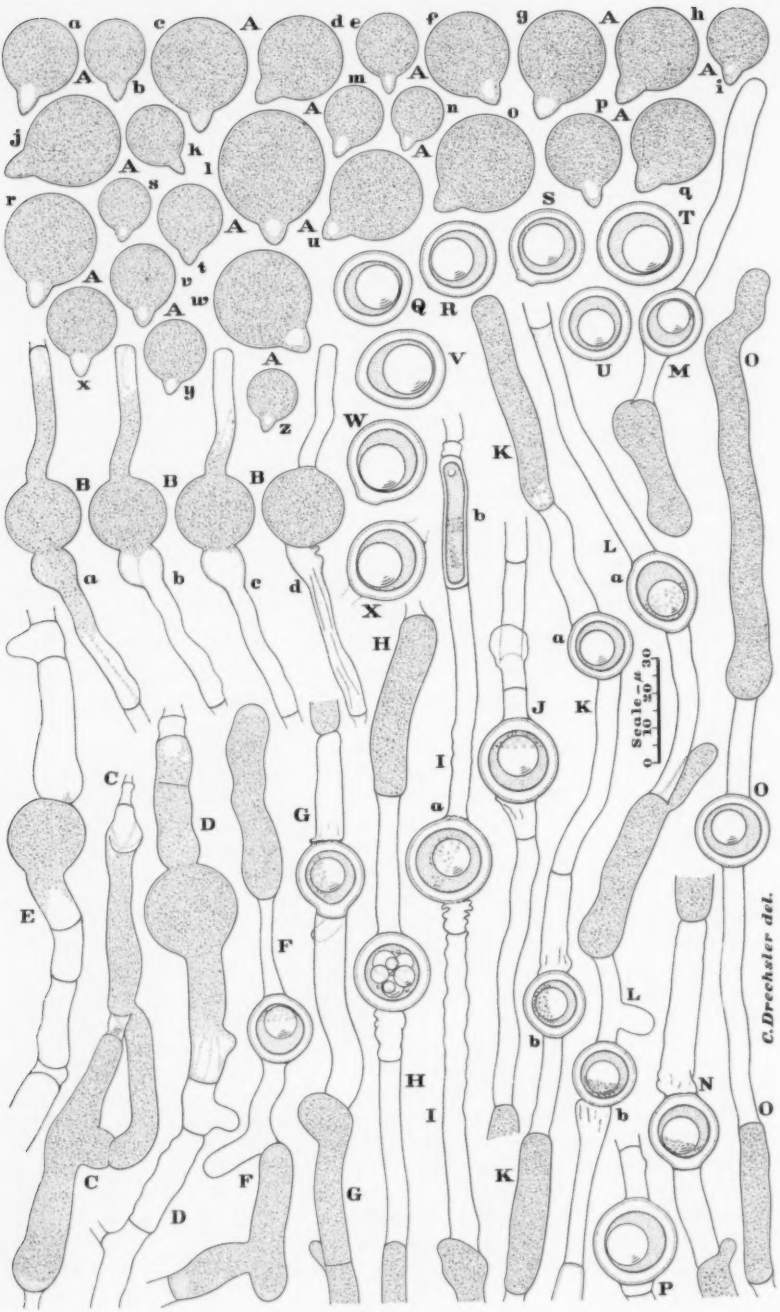

FIG. 8.

as well as scattered unicellular migratory hyphae (FIG. 7, H, *a–c*) that long continue to move through the substratum, steadily expending their limited substance in forming new tubular membrane.

The conidiophores produced abundantly in cultures of *Conidiobolus gonimodes* usually do not project into the air more than 50 μ. Sometimes the aerial shaft ascends from one end of the parent hyphal cell (FIG. 7, I) and at other times it ascends from a middle position (FIG. 7, J). Rather often it is noticeably distended 5 (FIG. 7, J) to 15 μ (FIG. 7, K) below the attachment of the young conidium, but often, again, it shows no pronounced swelling (FIG. 7, I, L, M). During the later stages in the migration of protoplasmic materials from the parent cell a conoidal partition is progressively pushed forward from the base of the conidium (FIG. 7, K). When migration of cellular contents has been concluded, the finished partition protrudes convexly upward (FIG. 7, L–O). In lima-bean-agar cultures somewhat branched conidiophores that bear a conidium on each branch (FIG. 7, O) are found here and there. Soon after being delimited the conidium springs off forcibly through sudden eversion of its basal membrane. A denuded conidiophore, whether simple (FIG. 7, P, Q) or branched (FIG. 7, R), in which considerable protoplasm is retained may put forth a new fertile branch

FIG. 8. *Conidiobolus gonimodes* drawn with the aid of a camera lucida; × 500. A, Detached conidia, *a–z*, showing variations in size and shape. B, Series of 4 stages, *a–d*, in conjugation between 2 adjacent hyphal segments, drawn at intervals of 20 minutes. C, Sexual reproductive unit in which 2 noncontiguous consecutive hyphal branches have been united by means of a lateral branch. D, Reproductive unit in which each of the 2 conjugating contiguous segments has formed at least 2 cross-walls. E, Reproductive unit showing 2 partitions in the empty tubular membrane of the receptive segment and 1 or possibly 2 partitions in the contributing segment. F, G, Portions of hyphae in each of which is intercalated a sexual reproductive unit that contains a mature zygospore and shows no cross-wall in the empty tubular membranes of the paired segments. H, Portion of hypha with an intercalated sexual reproductive unit that contains an approximately mature zygospore with plural reserve globules and shows the wrinkled membrane of the adjacent distention set off by a cross-wall. I, Portion of hypha bearing a sexual reproductive unit in which are included a mature zygospore, *a*, and a thick-walled cylindrical spore, *b*; the wrinkled membrane of the distention adjoining the zygospore is set off by a cross-wall. J, A mature sexual reproductive unit in which the empty tubular membrane of either segment shows a cross-wall about 10 μ from the ripe zygospore. K, L, Portions of hyphae that are each associated with 2 mature sexual reproductive units, *a* and *b*; in K the 2 units are separated by a cross-wall but no cross-wall divides the empty interior of any gametangium. M–O, Portions of hyphae associated with sexual reproductive units that each contain a mature zygospore; no cross-wall divides the empty interior of any gametangium. P, Mature zygospore with adjoining portions of empty hyphal membrane. Q–X, Mature zygospores showing usual variations in size and shape.

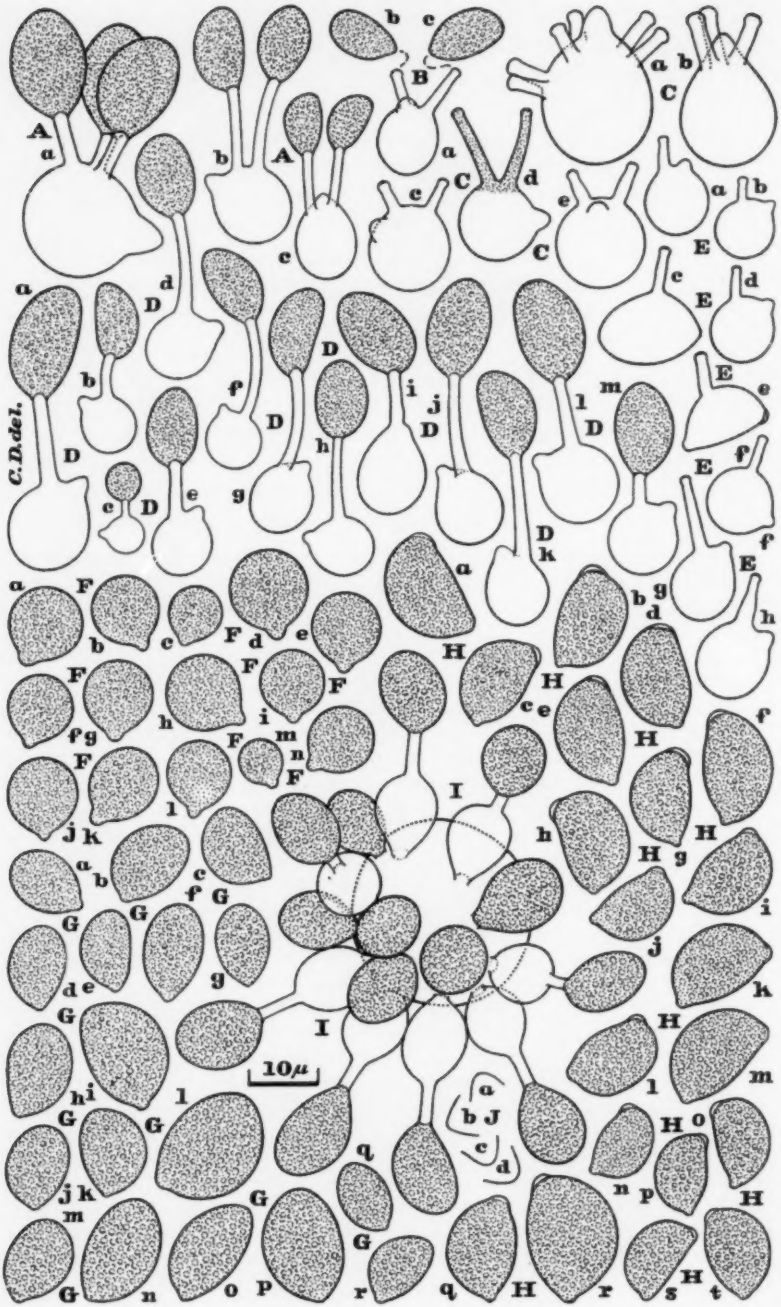

FIG. 9.

(FIG. 7, S) and give rise to an additional conidium. When an empty denuded conidiophore topples on to moist substratum, its tubular membrane may quickly vanish, so that afterwards only the distal wall remains as a visible remnant (FIG. 9, J, *a–d*).

The detached conidia (FIG. 7, T, *a–z*, U; FIG. 8, A, *a–z*) of *Conidiobolus gonimodes*, like those of *C. polyspermus* and *C. brefeldianus*, show an abruptly protruding basal papilla. On a fresh substratum they often put forth 1 or 2 germ hyphae that grow out into a new mycelium. On a substratum already occupied by the fungus they extend in many instances a short phototropic outgrowth (FIG. 7, *V–X*) that soon bears terminally a daughter conidium (FIG. 7, Y, *a–h*), which springs off forcibly, leaving behind the empty membrane of its parent (FIG. 7, Z). The epithet applied to the fungus is intended to be conveniently suggestive of its copious asexual reproduction.

The development of plural microconidia on nonphototropic spurs arising from detached conidia (FIG. 9, A, *a–c*) takes place more freely in *Conidiobolus gonimodes* than in the Florida isolate held referable to *C. brefeldianus*. As the microconidia (FIG. 9, B, *b, c*) usually spring off, even if only rather feebly, they are often found lying detached near the parent conidium (FIG. 9, B, *a*). The empty conidial envelopes (FIG. 9, C, *a–e*) usually collapse soon after they become denuded. In aging cultures where all the substratum is already occupied by the fungus, the detached microconidia commonly engage in prolonged repetitional development, each of them giving rise on a rather slender germ hypha to a new microconidium (FIG. 9, D, *a–m*), which springs off, leaving behind the denuded envelope of its parent (FIG. 9, E, *a–h*). In their successive generations the microconidia necessarily incur some reduction in size and usually also some haphazard changes in shape. Many are of a globose shape modified by a basal papilla (FIG. 9, F, *a–n*) usu-

FIG. 9. *Conidiobolus gonimodes* drawn with the aid of a camera lucida; × 1000. A, Globose conidia, *a–c*, that have given rise to plural microconidia. B, Empty conidium, *a*, with its progeny of 2 microconidia, *b* and *c*, lying nearby. C, Empty conidia, *a–e*, with plural stalks, denuded of microconidia, arising from them. D, Empty envelopes of microconidia, *a–m*, each with an empty stalk bearing aloft an elongated daughter microconidium. E, Empty microconidia, *a–h*, each with a stalk denuded of the daughter conidium it earlier supported. F, Microconidia, *a–n*, of globose shape modified at base by an abruptly protruding papilla. G, Microconidia, *a–r*, of elongated ellipsoidal shape with a usually small and rather variable protrusion at the base. H, Microconidia, *a–t*, of gibbous ellipsoidal form, each showing at its tip a cap of adhesive material. I, Globose conidium that produced 14 microconidia, which have remained attached although 8 of them have each given rise to a daughter microconidium. J, Conoidal membranous parts, *a–d*, each representing the distal wall of a conidiophore.

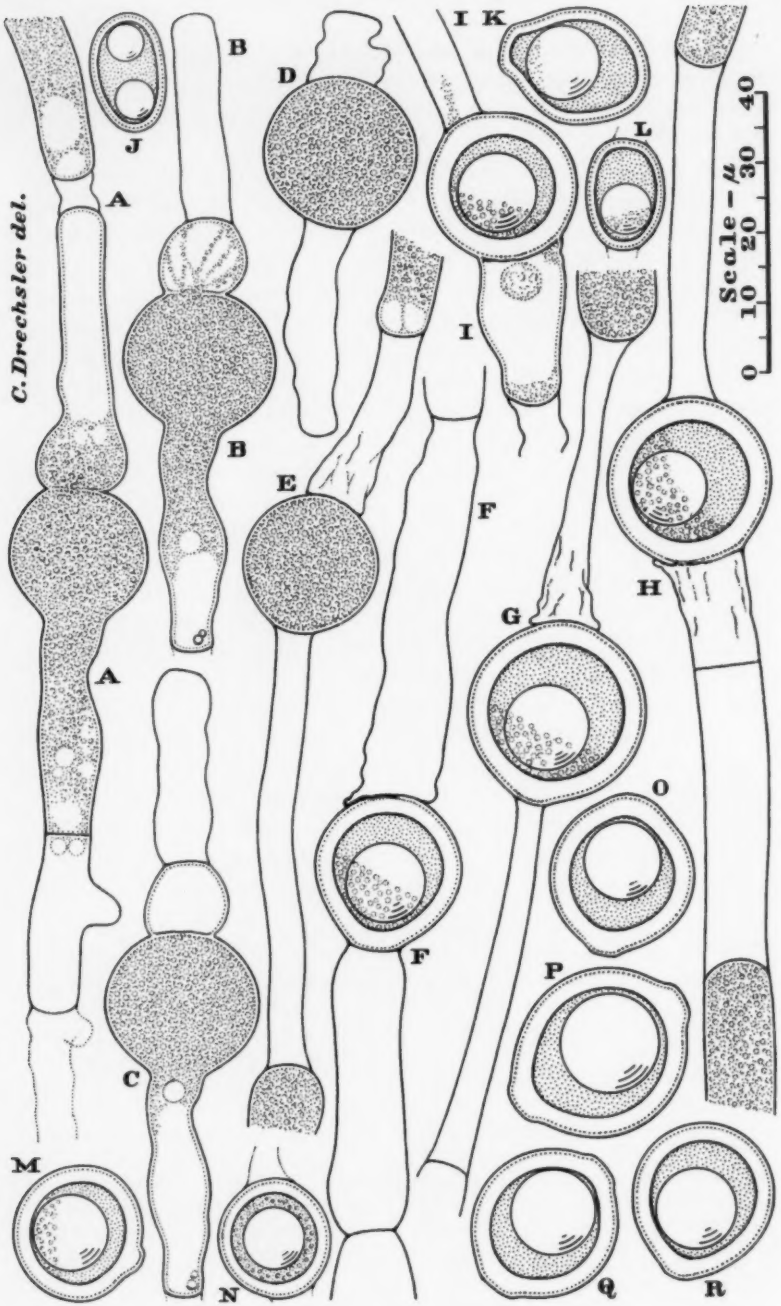

FIG. 10.

ally somewhat smaller proportionally than the papillae of most large conidia. Others (FIG. 9, G, *a–r*) present an elongated elliptical profile that in many instances is asymmetrical, being nearly semicircular on one side and less prominently curved on the other. The more pronouncedly asymmetrical or gibbous spores often are provided at the tip with a slightly protuberant cap (FIG. 9, H, *a–t*) consisting apparently of adhesive material. Nearly all unsymmetrical microconidia are borne more or less obliquely on their supporting stalks (FIG. 9, I), so that the adhesive cap in gibbous spores is exposed to contact laterally as well as distally.

Similar axially curved or gibbous spores in which an obliquely inclined distal portion bears an adhesive cap in a somewhat lateral position have been observed also in the repetitional development of microconidia in several congeneric fungi. In contaminated Petri plate cultures of *Delacroixia coronata* such spores are often produced with notable consistency by the numerous scattered microconidia that lie partly imbedded in a shallow layer of bacterial slime. Provision for exposing an adhesive apex to contact both laterally and distally would likewise seem present in secondary conidia of the elongate type produced by some members of the Entomophthorales. Thus, while in all species of *Basidiobolus* the proximal portion of the elongate secondary conidium appears usually in axial alignment with the straight slender germ conidiophore supporting it, curvature in the median and distal portions is often sufficient to tilt the adhesive beak well toward one side. Indeed, in a relevant figure given by Eidam (**1886**: Pl. 9, Fig. 16) in the original account of his *B. ranarum* the enlargement that presumably represents the elongated conidium of that taxonomically important fungus is shown obliquely oriented not only in its median and distal portions but throughout its length. In the diagnosis given by Thaxter (**1888**) under the binomial *Empusa* (*Triplosporium*) *fresenii* Nowakowski and also in the diagnosis of 3 fungi he newly described in *Empusa* under the epithets *lageniformis*, *geometralis*, and *occidentalis*, the distinctive almond-shaped secondary

FIG. 10. *Conidiobolus gonimodes* drawn with the aid of a camera lucida; × 1000. A–D, Four stages in conjugation of adjoining hyphal segments, the stages B–D being drawn after successive intervals of 20, 20, and 30 minutes, respectively; after a cross-wall had been formed in the receptive segment another was deposited in the contributing segment at the junction of the distention and the tubular portion of hypha. E, Sexual reproductive unit containing a young zygospore newly walled off. F–H, Sexual reproductive units each of which contains a mature zygospore; only the empty contributing segment in H is divided by a cross-wall. I, Mature zygospore with adjoining portions of hyphal membrane. J–R, Mature zygospores showing variations in size, shape, and internal structure.

conidia were set forth as being "borne obliquely on capillary conidiophores"; and although the term "obliquely" was omitted in his diagnosis of *Entomophthora sphaerosperma* Fresenius, his figures show the almond-shaped conidia of that species likewise in oblique posture.

When *Conidiobolus gonimodes* is grown on maize-meal agar containing some finely divided maize-meal in suspension, it gives rise in moderate number to units of sexual reproductive apparatus (FIG. 8, B, *a–d*; C; D; FIG. 10, C–D, E), which at maturity yield durable zygospores of correct internal structure (FIG. 8, F–H; I, *a*; J; K, *a, b*; M–X; FIG. 10, F–R). In some few instances 2 consecutive segments separated by an empty portion of tubular membrane conjugate by means of an arched lateral branch (FIG. 8, C), but much more often pairing takes place between 2 adjoining hyphal cells. As in *C. polyspermus* the adjoining cells here show their readiness for union by swelling at their abutting ends; and as in that species, again, one of the resulting distentions soon reaches definitive size, whereas the other keeps on increasing in volume (FIG. 8, B, *a–c*; D; E; FIG 10, A–C) until after receiving the protoplasmic contents of both cells it becomes walled off as a young zygospore (FIG. 8, B, *d*; FIG. 10, D, E). The ripe zygospore with its large reserve globule imbedded eccentrically in protoplasm of nearly homogeneous or slightly granuliferous consistency reveals the internal make-up most usual in the several species of *Conidiobolus* for which zygospores are known.

The progressive migration of protoplasm into the growing zygospore is often accompanied by deposition of 1 or 2 cross-walls in either or in both of the conjoined hyphal segments (FIG. 8, D, E; FIG. 10, A–D, H), though in many mature sexual units the empty tubular membrane of each segment shows no cross-wall other than the one originally delimiting it (FIG. 8, F, G; K, *a, b*; L, *a, b*; M–O; FIG. 10, E–G). In instances where a cross-wall in the contributing segment occurs at the junction of distention and unmodified hypha (FIG. 8, H; I, *a*; J; FIG. 10, C, D, H, I) the distention is walled off as a cell that in its position relative to the zygospore presents some outward parallelism with an antheridium in a monoclinous sexual unit of *Pythium ultimum*. This parallelism can hardly be considered important as it appears at a late stage in conjugation. A few mature reproductive units contain, in addition to the globose zygospore, a thick-walled cylindrical spore (FIG. 8, I, *b*) that may possibly be interpreted as a chlamydospore or, perhaps, as a supernumerary zygospore.

The zygospores of *Conidiobolus gonimodes* retain their viability for a long time. New growth of the fungus ensued consistently when transfers were made to newly slanted tubes of sterile maize-meal agar from

maize-meal-agar tube cultures that had been stored at 8° C for more than 4½ years and for approximately 3 years of this period had been in a completely air-dry state.

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BASIDIOSPORES OF SEVERAL SPECIES OF AGARICALES DISCHARGED AND COLLECTED IN PAIRS

E. B. Lambert and T. T. Ayers [1]

(with 4 figures)

While collecting spore prints of *Agaricus bisporus* (Lange) Sing. for single-spore isolations, we noticed that the spores appeared in pairs on the collecting surface. Since this phenomenon was unexpected in the light of Buller's classical studies, a series of tests were undertaken to learn whether pairing occurred regularly or only under very special conditions.

Most of these tests were made in small settling towers in which sporophores were suspended about 15 centimeters above the collecting surface. The sporophores were harvested when mature and secured in position in the towers with their gills or pores as nearly vertical as possible. The spore prints were collected on glass microscope slides and usually observed without cover slips under low power of a compound microscope. We soon learned that the identity of pairs of spores is obscured by crowding and overlapping when the spore prints are too dense. Pairs are clearly apparent, however, when only 20 to 40 spores are distributed on the glass slides within a low-power microscope field. In some instances a suitable spore density was obtained in 10 seconds, but in other cases as long as 5 minutes was required.

Whenever spore discharge was normal and abundant, several pairs of spores could be seen in each square millimeter of the collecting surface (Figs. 1–4). In one series of tests sporophores were suspended at different heights above the glass slides. In these tests paired spores were clearly evident on the slides even after the spores had settled through a vertical distance of 1 meter. We interpreted this as evidence that each pair came from the same basidium and made the journey together to the collection surface. The paired spores must be discharged from the basidium simultaneously or at most within $\frac{1}{10}$ second of each other. Otherwise, how can they fall in slightly turbulent air

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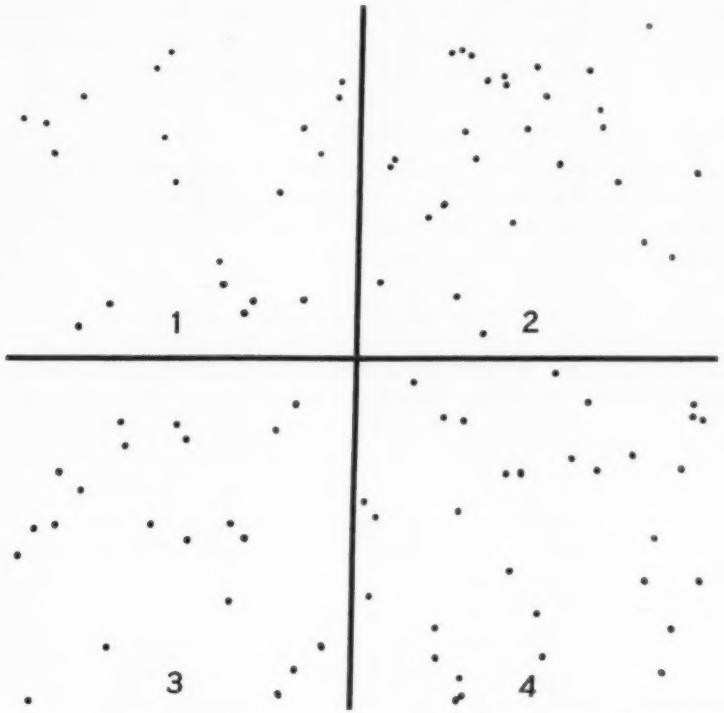
through a distance ten thousand times as great as the distance between the paired spores and arrive on the collection surface separated by only 5 or 10 spore diameters?

Spore prints were observed from 7 species of the Agaricaceae and 3 of the Boletaceae. Paired spores were obtained in all of the species



FIGS. 1–4. Patterns of spore distribution in dilute spore prints collected from 4 species of agarics. 1. *Amanita phalloides*. 2. *Lactarius piperatus*. 3. *Agaricus bisporus*. 4. *Galera tenera*. The figures are made from photomicrographs of spore prints (× 55) with the position of each spore indicated by an ink dot.

studied, which included: *Agaricus bisporus* (Lange) Sing., *Agaricus campestris* L. ex Fr., *Amanita phalloides* (Vaill. ex Fr.) Secr., *Galera tenera* (Schaeff. ex Fr.) Quél., *Lactarius colorascens* Pk., *Lactarius piperatus* (L. ex Fr.) S. F. Gray, *Lepiota lutea* Quél., *Boletus bicolor* Pk., *Boletus edulis* Fr., and *Boletus scaber* Fr. Thus, it seems probable that many species throughout the Agaricales may discharge spores in

pairs. In spore prints from 4-spored species, the spores frequently appeared on the slides in groups of 3 but rarely in groups of 4.

Since the spores in a pair are deposited under identical conditions, they tend to germinate at the same time. Simultaneous germination of pairs of spores of *Agaricus bisporus* collected on the surface of an agar medium in petri plates was observed. We anticipate that it will be much easier to isolate both sporelings in these pairs than to obtain paired sporelings by the usual procedure of picking the spores separately off the basidium and taking a chance on their both being viable.

SUMMARY

In sparse spore prints of several species of Agaricales, the basidiospores were observed in pairs. This deposition of spores in pairs is interpreted as evidence that spores are frequently discharged simultaneously from individual basidia and fall together to the collection surface.

ACKNOWLEDGMENT

The writers gladly acknowledge their indebtedness to J. A. Stevenson for identifying most of the species studied.

ENTOMOPHTHORA CORONATA, THE ETIOLOGIC AGENT OF A PHYCOMYCOSIS OF HORSES

CHESTER W. EMMONS AND CHARLES H. BRIDGES

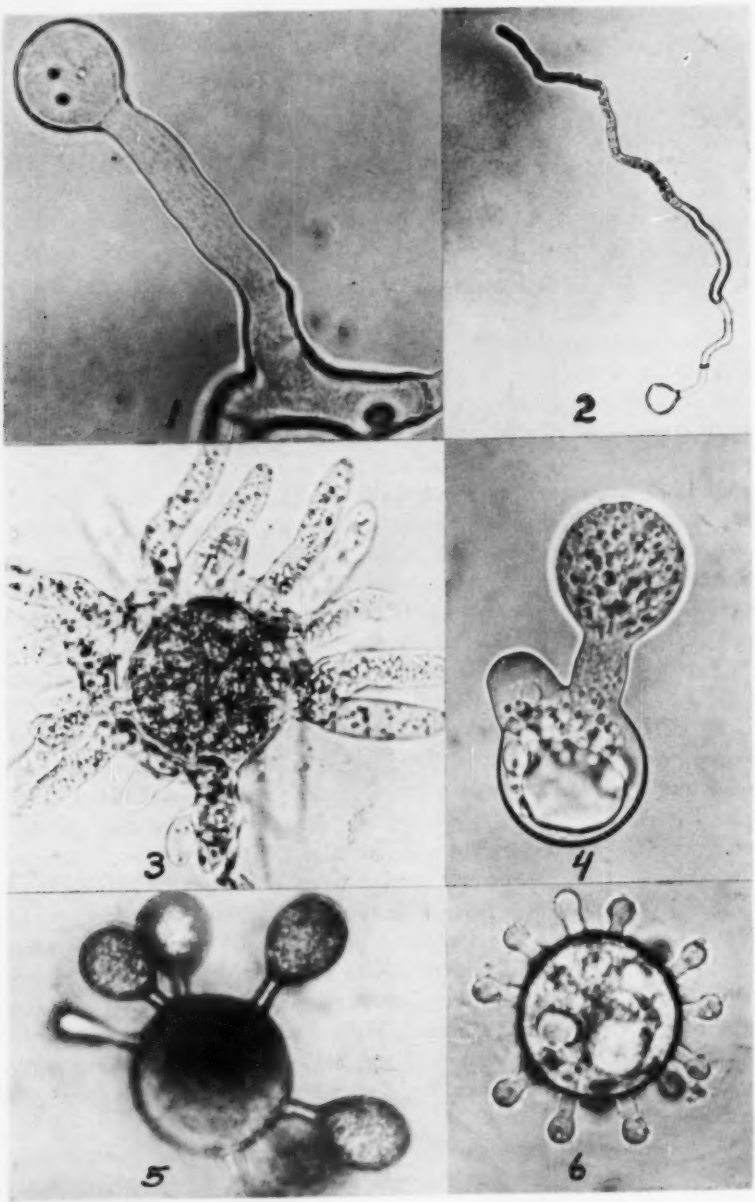
(WITH 10 FIGURES)

Mycologists are generally acquainted with species of *Entomophthora* as parasites of insects. In a separate paper (1) we are reporting four cases of a chronic granulomatous disease of the nasal mucosa and the lips of horses caused by *Entomophthora coronata* (Costantin) Kevorkian (4). Germination of conidia of this fungus may be by a single hypha, by multiple hyphae, by replication of a single conidium on a short conidiophore, or by replication of many conidia on many short conidiophores. In many cultures all conidia are covered by hair-like appendages. The versatility of this fungus, the taxonomic significance of this variability, and the proof that *E. coronata* can parasitize mammalian tissues should be of interest to mycologists.

The fungus was easily demonstrated in the tissues of the host where it grew as a coenocytic mycelium.

In culture *E. coronata* grows rapidly. A subculture on glucose-neopeptone agar incubated at 30°C begins to discharge spores in 10–15 hours and the colony reaches a diameter of 6 cm within 48 hours. The colony is colorless to yellowish white. It is at first flat and glabrous but it quickly produces radial and irregular folds and a white bloom on the surface which represents surface hyphae and short conidiophores (FIG. 1). The colony remains membranous without deep penetration of the agar substratum and without production of a conspicuous aerial mycelium. In a petri dish incubated in an inverted position and in agar slants incubated vertically, the glass surface opposite the colony is covered in 24–36 hours by a film of conidia which have been discharged forcibly from the tips of conidiophores and which germinate by production of hyphae or reproduce by replication on the nonnutritive glass surface (FIGS. 2–7).

Hyphae vary in dimensions between 6 and 15 μ. They contain droplets and granules of varying size which apparently are fat, glycogen and other metabolites. Many germinated spores and older hyphae appear



FIGS. 1-6.

to be completely emptied of protoplasm and these empty structures are separated by septa from viable portions of hyphae.

Conidiophores emerge from engorged hyphal segments at or near the surface of the medium. They are phototropic and discharge conidia forcibly toward a light source. They are 8–12 × 60–90 μ, bulge slightly in the midportion and taper toward the tip (FIG. 1). The protoplasm is vacuolate with many granules and particles which often show a linear arrangement.

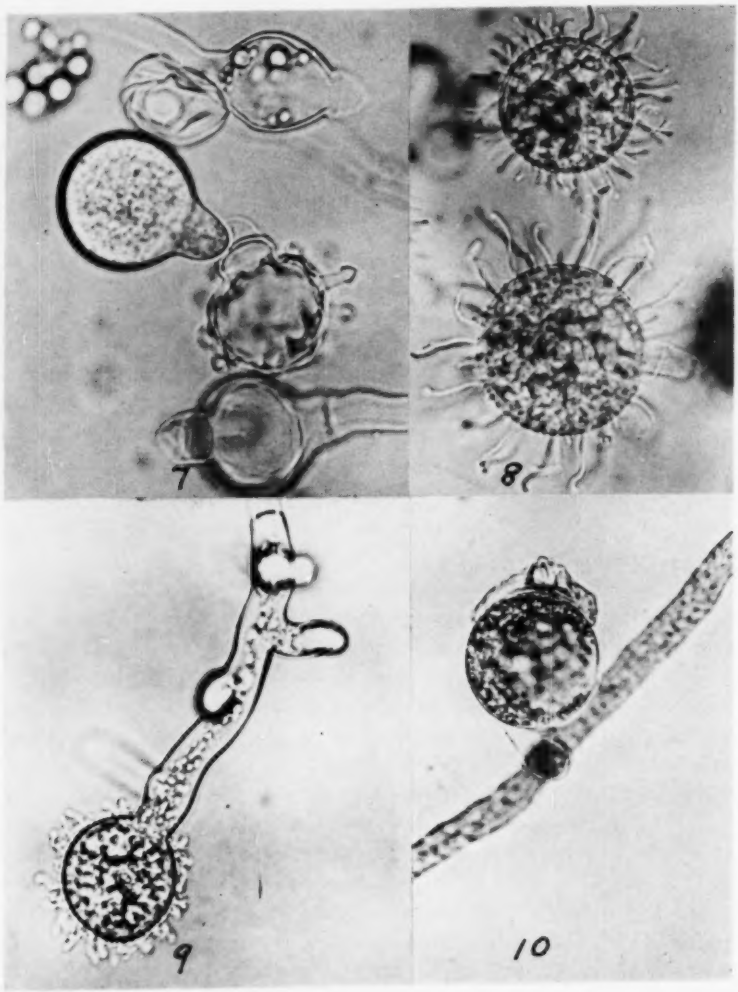
The conidium originates as a spherical enlargement at the tip of the conidiophore from which it eventually is separated by a double wall. The tip of the conidiophore extends into the young spore as a columella which is rigid and persistent. At maturity the spore is forcibly discharged by separation of the component parts of the double wall and sudden eversion of the spore wall at the point where it has been invaginated by the tip of the conidiophore. This everted portion of the wall persists as a prominent papilla at the base of the discharged spore. G. W. Martin, who described this species under the name *Conidiobolus villosus*, stated that the spore may be thrown vertically 25–30 mm and laterally 30 mm (**2**).

Conidia are spherical except for a conspicuous basal papilla which marks the former point of attachment to and discharge from the conidiophore (FIG. 7). Diameters of the primary spores are 36–44 μ. Secondary spores formed by replication are smaller.

In some cultures of each of the four strains isolated from lesions of horses and in a strain isolated from soil in Australia by Dr. E. Beatrix Durie, some of the conidia extrude through pores in the wall a material which forms hair-like appendages over almost the entire spore surface (FIG. 8, 9). These are brittle and they may break loose from the spore and into fragments when spores are suspended in fluid for microscopic examination. They are like the structures described by Martin in *Conidiobolus villosus* (**2**). Although brittle and apparently noncellular, they may be related to the type of multiple germination by replication frequently observed in this fungus.

Conidia which fall after discharge upon a clean glass surface may sporulate by replication. That is, the conidium produces a short ger-

FIGS. 1–6. *Entomophthora coronata*. 1. Conidiophore and immature conidium. × 690. 2. Germination of conidium with expulsion of protoplasm from spore and from adjacent cells of germination tube. × 212. 3. Germination by multiple hyphae. × 690. 4. Sporulation by replication on glass surface. × 690. 5. Multiple germination by replication. × 690. 6. Multiple germination by replication. × 690.



FIGS. 7–10. *Entomophthora coronata*. 7. Conidium showing prominent papilla and spent conidium which has produced multiple secondary conidia. × 690. 8. Old conidia with hair-like appendages. × 690. 9. Germination of conidium with appendages. × 690. 10. Zygospore (?). × 690.

mination tube which becomes at once a conidiophore and bears a secondary conidium which is forcibly discharged. This sporulation by replication may continue (the progeny becoming progressively smaller) until the stored food supply is exhausted or until a spore falls upon a nutrient

substratum. A conidium may produce many short conidiophores and small secondary conidia simultaneously (FIGS. 5–7). These multispored conidia may germinate on a glass surface among ungerminated spores, those which have produced single conidiophores and those which have produced long hyphae. It seems probable that multiple sporulation of this type is related to the same conditions of spore metabolism and wall structure that are associated with the appearance of hair-like appendages.

Germination of some conidia on moist glass surfaces and those which fall on nutrient agar may be by single or multiple germination tubes. Formation of multiple tubes is compatible with the multinucleate condition of this species reported by Martin (2) and of other species of *Conidiobolus* reported by other investigators.

Smooth thick-walled spores slightly smaller than the average size of primary conidia are found in some cultures on Sabouraud's agar and when a large inoculum is placed on a medium such as cornmeal agar (FIG. 10). They resemble zygospores but we have not yet observed with certainty the manner in which they originate.

Three of the strains of *Entomophthora coronata* isolated from nasal granulomata of three horses were similar when first isolated but now differ in several characteristics. The fungus from Case 1 now grows slowly, is inhibited by temperatures above 33°C, and sporulation is sparse and delayed. The fungi from Cases 2 and 3 grow more rapidly than that from Case 1, grow well at 40°C and the former sporulates frequently by production of multiple secondary conidia (FIG. 5). The one from Case 4 is a recent isolate which has been studied less intensively. We believe these differences are related to mutations which have occurred while the fungi have been maintained in culture in the laboratory and that this ready mutability must be considered in any taxonomic consideration of species.

This fungus was first described by Costantin (3) as *Boudierella coronata* in 1897. Since the genus name *Boudierella* had been used previously, Saccardo and Sydow transferred the species to *Conidiobolus,* but the generic name *Conidiobolus* has been used for members of the Entomophthoraceae which were saprophytic. It is apparent from Kevorkian's isolation of this fungus and from experimental infection of termites and from our isolation of it from nasal granulomata in horses that it is capable of parasitism. Since the commonly used name *Empusa* is invalid because of its previous use as a genus of orchids, we follow Kevorkian in placing the fungus in the genus *Entomophthora,* using the earliest known specific name and the combination proposed by Kevorkian, *Entomophthora coronata.*

SUMMARY

Entomophthora coronata was isolated from granulomatous lesions in the nasal mucosa and skin of four horses. The fungus grew in the tissues in the form of coenocytic hyphae. In culture *E. coronata* conidia germinated by production of single or multiple germination tubes, or by replication of single or multiple secondary conidia. Many of the conidia, which are multinucleate, produce hair-like appendages.

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REVIEWS

LICHEN HANDBOOK. A GUIDE TO THE LICHENS OF EASTERN NORTH AMERICA, by Mason E. Hale, Jr. x + 178 p., 20 pl., 58 fig. Smithsonian Institution, Washington, D. C. 1961. Price, $4.00.

This handbook meets a long-standing need for a modern treatment of the lichens, supplanting the out-of-print Schneider Textbook. The text contains chapters on morphology and anatomy, reproduction, physiology, symbiosis, chemistry including identification of lichen acids and chemical strains, economic uses, phytogeography, and classification, a glossary, and a bibliography of 8 pages. A chapter on collection and identification includes a key to alphabetically arranged genera of the fruticose and foliose lichens, and within each genus keys to the species. Crustose species are keyed to genera only. The diagnostic characters emphasized are macroscopic except in the key to crustose genera. There is a useful list of corrections for the names used in Bruce Fink's recently reprinted *Lichen Flora of the United States*, the only available comprehensive work on lichens in America.

Amateur and professional botanists will find this an excellent introduction to a field of study that has experienced a tremendous impetus since the War, and that has been handicapped by lack of an adequate and modern introduction to this group of plants.—JOSIAH L. LOWE.

DIE PHYTOPATHOGENEN GROSSPILZE DEUTSCHLANDS, by Hanns Kreisel. 284 p., 111 figs. Gustav Fischer, Jena, 1961. Price, 33.20 DM.

Dr. Kreisel indicates that this book has been prepared for the plant pathologist, forester, mycologist and amateur concerned with the study of the large fungi which parasitize or are associated with living and dead trees and shrubs, grasses and cultivated plants. By large fungi the author means Basidiomycetes exclusive of the rust and smut fungi.

The first part (35 p.) provides general information on morphology (with line drawings) and terminology of Basidiomycetes as well as phytopathological information on parasitism, wood decay, fairy rings, weed fungi, hosts, control methods, etc.

The remainder of the book includes keys to major groups of Basidiomycetes, to genera and to the species known to occur in Germany with brief description of the orders, families, genera, and species. For the more important pathogens detailed discussions are given. Good line drawings of structures are inserted in the keys and at appropriate places in the text; maps showing the distribution of several species in Germany are included.

Nomenclature and taxonomy are modern and follow recent works of European mycologists. After a host index, bibliography of 133 citations, an index of fungus taxa, and an index of German names of the fungi, 66 black and white photographs of the fungi are included. Most of these show the fungi in their natural habitat. For the most part the photographs are good.

Although written primarily for Germans, the book should prove useful to others in Europe and in America.—CLARK T. ROGERSON.

CHAMPIGNONS COMESTIBLES ET VENENEUX, by Marcel Locquin and Bengt Cortin. 156 p., illus. Fernand Nathan, Paris. 1959.

A HANDBOOK OF MUSHROOMS, by A. Pilát and O. Ušak. 91 p., 94 pls. col. Spring Books, London NW 5. ?1960. Price, approx. $1.50.

After the appearance of A. H. Smith's "The Mushroom Hunter's Field Guide," published by the University of Michigan Press in 1958, two European mushroom books of a similar kind have appeared to guide the amateur in his first steps. What all three books have in common is more than meets the eye at first glance. In the first place, particular attention is given to the illustration of the most important species. In the second place, the text is reduced to the minimum and contains only the most basic information. Thirdly, all three books are true pocket books and can be carried in the field. And finally, all three authors (respectively senior authors) are highly experienced well-known mycologists in their respective countries whose competence is beyond any question, and whose instructions are dependable and responsible. Even so, the beginner who has all three books in front of himself, will be bewildered by the fact that the same *Lactarius piperatus* which is—correctly—called edible by Smith and Pilát is called not edible by Locquin. The same *Boletus luridus* on which Smith comments "My advice . . . is that it is better to be safe than sorry" (the present reviewer has been sorry once!), is called a good edible mushroom by Pilát although with the correct qualification that "some mycologists consider

it poisonous when raw"; unfortunately, Locquin calls *Boletus luridus* edible without further comment. As for the classification and scientific nomenclature, we are already used to a somewhat more conservative yet still widely diverging set of names in the works of different authors. In this regard Pilát has come perhaps to the best solution by impartially indicating both correct and traditional, modern and antiquated names.

Pilát wrote a text which consists almost entirely of descriptive data on the fungi illustrated. This text is adequate and as clear as it is correct.

On the other hand, Locquin remains within the French tradition offering the usual chapters on general classification and his own version of mushroom classification which is somewhat halfway between Fries and Moser but not quite Heim or Kühner & Romagnesi either. The descriptive data are given in key form with many alternatives and although they are an ingenious digest of essential characters of the most important species, it remains somewhat doubtful whether a *Russula* can be determined this way. There is also the traditional chapter on types of poisoning and on the family mushroom growing of *Agaricus bisporus*, preceded by a culinary chapter which I find particularly attractive since I have tried out some of the recipes given and find them delicious.

Since the relatively restricted text puts all the emphasis on the figures, it is very important that those be well enough executed and reproduced to speak for themselves. I am inclined to think that Pilát and Ušak have, along with the more extensive and enchanting Japanese mushroom book by Imazeki and Hongo (recently reviewed in Mycologia **52**: 827), come to a very high degree of both scientific exactitude and artistic perfection in colored mushroom plates. Ušak (who, I believe, is the author of the plates) has been able to capture—beyond the diagnostic characters—the essentially particular in each species.

Locquin's book is also well illustrated. The attempt to express the preferred habitat of each species on the illustration itself is commendable. A minor omission: No illustration is given or mention made of the black truffles (*Tuber*). This, however, may have something to do with an otherwise very welcome trend in this kind of publication: International cooperation. It is a curious coincidence that the book written in English and published in London was edited and printed in Czechoslovakia. The book written in French and published in Paris was written under co-authorship of a Swede, is illustrated by paintings of a Swedish artist—or so I understand—, was first published by Gebers Forlag, Stockholm, and has now been printed in Holland.

The two pairs of authors should be congratulated on their mushroom

books. Both will be useful to the mushroom hunter and beginner.—ROLF SINGER.

MICHAEL/HENNIG HANDBUCH FÜR PILZFREUNDE; ZWEITER BAND, NICHTBLATTERPILZE, revised by Bruno Hennig. vi + 328 p., 26 figs., 120 colored pl. Gustav Fischer, Jena, 1960. Price, 38.70 DM bound.

Volume 2 of Michael's "Führer für Pilzfreunde" as revised by Bruno Hennig treats fungi exclusive of agarics. The text is in German.

Eight brief introductory chapters give explanations with line drawings of technical terms, a discussion of mycorrhiza, abnormal formation of fruitbodies, and fungus galls, a history of the discovery of fungus spores by Micheli and others, the life-work of J. C. Schaeffer, an extensive list of references useful in identification of fungi except agarics, a list of specialists for different fungus groups in central Europe, and a list of culture collections.

In the following 19 chapters characteristics, classification and general information of the major groups, families and genera, as well as brief descriptions of common species are presented. Except for a key to the earthstars of central Europe, keys are lacking. Nomenclature appears to be modern.

The major portion of the book includes colored illustrations with descriptions facing each plate of 285 fungi (27 boletes, 46 polypores, 15 thelephoraceous fungi, 26 hydnums, 25 clavarias, 4 stinkhorns, 23 puffballs and allied fungi, 13 hypogeous basiomycetes, 13 tremellaceous fungi, 66 discomycetes, 11 pyrenomycetes, 9 truffles, and 8 myxomycetes). Most of the colored illustrations are good; some, particularly of the boletes, clavarias and larger fungi, are excellent; several, particularly of the small discomycetes and slime molds, are poor.

Since the majority of the species described and illustrated are known to occur in America, the book should be useful to all amateur and professional mycologists.—CLARK T. ROGERSON.

ANNOUNCEMENTS

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(*Continued*)

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(Continued)

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The volume on North American Cup-fungi (Operculates), supplemented edition, by Dr. Fred J. Seaver, Curator Emeritus, The New York Botanical Garden, which has been out-of-print, is available in reprinted form. Copies of Dr. Seaver's North American Cup-fungi (Inoperculates) also are available. Both can be obtained from Hafner Publishing Co., Inc., 31 East 10th Street, New York 3, New York. Operculates, $15.00; Inoperculates, $10.00; special set price for both $22.50.

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Brittonia, quarterly; beginning in 1937, official publication of the American Society for Plant Taxonomists. $7.50 a year.

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